

JOURNAL OF ANATOMY

ORIGINALLY THE JOURNAL OF
ANATOMY AND PHYSIOLOGY

QL
801
J7

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY
OF GREAT BRITAIN AND IRELAND BY

J. S. BAXTER

G. A. G. MITCHELL

D. V. DAVIES

K. C. RICHARDSON

F. GOLDBY

E. W. WALLS

W. J. HAMILTON

J. D. BOYD (EDITOR)

VOLUME 90

JANUARY 1956—OCTOBER 1956

CAMBRIDGE
AT THE UNIVERSITY PRESS
1956

*Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)
and published by the Cambridge University Press
London: Bentley House, N.W.1
American Branch: New York
Agents for Canada, India, and Pakistan: Macmillan*

CONTENTS

PART 1—JANUARY 1956

	PAGE
The relation between the Schwann cell and the axon in peripheral nerves. By G. CAUSEY and H. HOFFMAN	1
Growth changes in the diameter of peripheral nerve fibres in fishes. By P. KYNASTON THOMAS	5
An experimental study of the secondary olfactory connexions in <i>Testudo graeca</i> . By H. J. GAMBLE	15
Terminal degeneration in the diencephalon after ablation of frontal cortex in the cat. By J. AUER	30
Corticofugal fibres to the brain-stem reticular formation. An experimental study in the cat. By GIAN FRANCO ROSSI and ALF BRODAL	42
The growth of the foetal skull. By E. H. R. FORD	63
The structure and development of cranial and facial sutures. By J. J. PRITCHARD, J. H. SCOTT and F. G. GIRGIS	73
The relationship between the pattern of ossification and the definitive shape of the mesosternum in man. By G. T. ASHLEY	87
Transitional epithelium and osteogenesis. By F. R. JOHNSON and R. M. H. McMINN	106
Ribonucleic acid-alkaline phosphatase distribution in the developing teeth of the rat. By N. B. B. SYMONS	117
The early stages of vaginal development in the sheep. By D. BULMER	123
Histochemistry of the rabbit placenta. By J. DAVIES	135
The ductus venosus of the pig. By A. D. DICKSON	143
The levator palati muscle. By R. FRANCE ROHAN and L. TURNER	153
REVIEWS	155

PART 2—APRIL 1956

Neural pathways in lactation. By J. T. EAYRS and R. M. BADDELEY	161
Some characteristics of myelinated fibre populations. By T. A. QUILLIAM	172
A note on terminal degeneration in the hypothalamus. By W. M. COWAN and T. P. S. POWELL	188
A study of silver degeneration methods in the central nervous system. By D. H. L. EVANS and L. H. HAMLYN	193
The immediate effects of ligature of vasa nervorum. By MICHAEL J. BLUNT and KATHLEEN STRATTON	204

	PAGE
The functional significance of the pattern of innervation of the muscle quadratus labii superioris of the rabbit, cat and rat. By RUTH E. M. BOWDEN and Z. Y. MAHRAN	217
Observations on the silver impregnation of nerve fibres in teeth. By R. W. FEARNHEAD and J. E. LINDER	228
Observations on the postural mechanism of the human knee joint. By J. W. SMITH	236
The evolution of the mammalian eardrum and tympanic cavity. By C. C. D. SHUTE	261
The development of the circulation in the spleen of the foetal rabbit. By O. J. LEWIS	282
Anatomical features of the human renal glomerular efferent vessel. By J. P. SMITH	290
The distribution of haemopoietic foci in the infantile human liver. By JOHN L. EMERY	293
The representation of skull shape by contour drawing. By D. I. G. BUNN and P. TURNER	298
A rapid method of graphic reconstruction. By C. H. BARNETT	304
REVIEWS	307

PART 3—JULY 1956

Arterio-venous anastomoses in the human external ear. By M. M. L. PRICHARD and P. M. DANIEL	309
Observations on the blood supply of the rabbit's ear and on the experimental new-formation of arterio-venous anastomoses. By BRUNO ROSSATTI	318
Observations on the capillary blood vessels of the human nail fold. By E. W. WALLS and T. J. BUCHANAN	329
On the individual variability of fibre composition in human peripheral nerves. By J. TOMASCH and W. A. BRITTON	337
Degeneration in the post-commissural fornix and the mammillary peduncle of the rat. By R. W. GUILLERY	350
Experimental studies of the vermal cerebellar projections in the brain stem of the cat (fastigiobulbar tract). By DONALD M. THOMAS, RONALD P. KAUFMAN, JAMES M. SPRAGUE and WILLIAM W. CHAMBERS	371
A study of the development of the cerebral cortex of the foetal guinea-pig by means of the ultra-violet microscope. By ARTHUR HUGHES and LOUIS B. FLEXNER	386
The early development of the golden hamster (<i>Cricetus auratus</i>). By W. J. HAMILTON and the late D. M. SAMUEL	395
The effect of cortisone acetate on the response of the regional lymph node to a skin homograft. By R. J. SCOTHORNE	417
Some morphological effects of large doses of cortisone in the rabbit with special reference to the thymus and appendix. By E. J. FIELD	428

	PAGE
The histochemical distribution of 'lipase' in the alimentary tract and associated glands of laboratory animals. By B. F. MARTIN	440
IN MEMORIAM: DR WYNFRID LAWRENCE HENRY DUCKWORTH	455
REVIEWS	457
BOOKS RECEIVED	460

PART 4—OCTOBER 1956

The development of the penile urethra in the pig. By T. W. GLENISTER	461
The uptake of radioactive sulphate by cells, fibres and ground-substance of mature and developing connective tissue in the adult mouse. By A. GLÜCKSMANN, ALMA HOWARD and S. R. PELC	478
The blood supply of the optic nerve and chiasma in man. By E. J. STEELE and M. J. BLUNT	486
A quantitative study of the postnatal changes in the packing density of the neurons in the visual cortex of the mouse. By M. HADDARA	494
The ultrastructure of the synaptic area in the superior cervical ganglion. By G. CAUSEY and H. HOFFMAN	502
The development of a compensatory collateral circulation to nerve trunk. By MICHAEL J. BLUNT and KATHLEEN STRATTON	508
The organ of Jacobson. By V. E. NEGUS	515
The occurrence of a middle superior alveolar nerve in man. By M. J. T. FITZGERALD	520
The arrangement of the ansa spiralis of the ox colon. By R. N. SMITH and G. W. MEADOWS	523
The development and fate of the abdominal chromaffin tissue in the rabbit. By REX E. COUPLAND	527
The role of the peritoneum in the formation of the septum recto-vesicale. By P. H. S. SILVER	538
Cornification of the human vaginal epithelium. By A. W. ASSCHER, C. J. TURNER and C. H. DE BOER	547
The aberrant renal artery. By F. T. GRAVES	553
REVIEWS	559
PROCEEDINGS	561
INDEX TO VOLUME 90	603
SUPPLEMENTARY INDEX OF PROCEEDINGS	607

THE RELATION BETWEEN THE SCHWANN CELL AND THE AXON IN PERIPHERAL NERVES

BY G. CAUSEY AND H. HOFFMAN*

Department of Anatomy, Royal College of Surgeons of England

The very close relationship between the Schwann cell and the nerve cell process has been long recognized. Nageotte (1932) sums up the position: 'It (the Schwann cell) maintains singularly close anatomical and physiological relations with the neurite but, in this synthesis, the two protoplasts always remain distinct.' The nomenclature, however, as applied to the optical demonstration of stained sections, is by no means clear, its complexities have been discussed fully by Young (1942).

Electron-microscopic examination of thin sections has further emphasized this very close relationship. Gasser (1952) demonstrated the non-myelinated fibres running in the cytoplasm of the Schwann cell and connected to the surface of the Schwann cell by a 'mesaxon'. Hess & Lansing (1953) showed small myelinated fibres running in the Schwann cytoplasm, and Causey & Hoffman (1954) demonstrated these relationships in both normal and regenerating material. Geren (1954) reported a detailed examination of the relationship between the Schwann cell membrane and the myelin in the chick embryo. Gasser has recently published (Gasser, 1955) an extensive investigation of the structure and function of non-myelinated fibres in the dorsal roots of the cat.

The present paper is concerned particularly with the relationship of the Schwann cell and the non-myelinated fibres in normal nerves of the rat.

MATERIAL AND METHOD

The material illustrated is taken from the vagus nerve in the neck and from the lumbar dorsal roots of adult rats, anaesthetized with Nembutal. The material was fixed for 4 hr. in 1% osmium tetroxide, with veronal buffer pH 7.2-7.4 at 4°C. Embedded in methacrylate and cut at 200-400 A.U. The methacrylate was not removed, and the specimens were examined in a Metropolitan Vickers EM4, with 50 μ objective aperture.

RESULTS

In the normal vagus nerve the myelinated fibre is surrounded by a sheath of Schwann protoplasm. This sheath may be very thin in the parts of the internode between the Schwann nucleus and the node of Ranvier, such a region is seen in a myelinated fibre in Pl. I, fig. 1. Throughout the length of the Schwann cell it is surrounded by a double-layered limiting membrane, which surrounds and delimits the whole axon, myelin, Schwann cell complex. As Cajal (1909) said: 'La membrane de Schwann doit être considérée, au point de vue histologique comme une véritable membrane cellulaire.'

* Proffit Research Fellow of the Royal College of Surgeons of England.

Between the myelinated fibres are bundles of non-myelinated fibres. There are single fibres, but most are aggregated together in collections of anything up to twelve or fourteen fibres, but whether there be one fibre or many the whole is contained within the cytoplasm of a Schwann cell. The area between the Schwann cell units, whether these contain one myelinated fibre, one non-myelinated fibre or a number of non-myelinated fibres, contains only bundles of collagen fibres.

The same general relations are seen in the dorsal root (Pl. 2, fig. 4) except for two points. First, the bundles of non-myelinated fibres, although contained in similar Schwann cytoplasm units, are less distinctly demarcated from each other and secondly, and probably also explanatory of the first, there is apparently less collagen between the bundles. In both Pl. 1, fig. 1, and Pl. 2, fig. 4, the double structure of the limiting membrane is seen, with the two electron dense lamellae.

Some of the detail of the relationship between the non-myelinated fibres and the Schwann cells is shown in figs. 2-6. Fig. 2 shows the axon in a depression on the surface of the Schwann cell, the fibre is incompletely enveloped by the cell membrane and there is no mesaxon: it seems probable that this appearance is associated with the fibre entering or leaving a Schwann cell. In Pl. 1, fig. 3, invagination is complete, but the mesaxon is short. The double-layered membrane around the axon is made up of one lamina continuous round the axon and the other formed by the inner layer of the double limiting membrane of the Schwann cell. The mesaxon is formed by the juxta-position of the invaginated inner layer of the Schwann limiting membrane, the continuity of the outer layer of the cell membrane being re-established. Pl. 2, fig. 5, shows a longer, two-layered mesaxon from the Schwann surface to the small nerve fibre. Here again the two laminae of the mesaxon are resolved.

In Pl. 2, fig. 6, the two-layered mesaxon has formed a second double layer round the nerve fibre. The complexity of these attachments becomes more marked when a number of the fibres are held close together within one Schwann cell, as can be seen in Pl. 1, fig. 1, but the basic pattern of a Schwann cell with two dense layers in the cell membrane, an axon with two dense layers in its limiting membrane and a mesaxon consisting of two dense layers only, is observed constantly.

DISCUSSION

The intimate enfolding of all the nerve fibres within a Schwann cell cytoplasm seems to be quite constant. It might be expected that when a nerve fibre is enfolded by or invaginates a Schwann cell a fold in the Schwann cell membrane would give rise to four electron dense laminae, but this fourfold mesaxon has never appeared in our material, there always seems to be a reformation of the outer layer of the Schwann cell membrane leaving only the inner layer thereof involved in the mesaxon. This outer layer has been called by Gasser (1955) a basement membrane. Geren (1954) also shows a double-layered mesaxon, but suggests that this attachment is formed in the chick embryo from a single-layered Schwann cell membrane. An observation that is perhaps pertinent to this subject has been reported elsewhere (Causey & Hoffman, 1955) in that, when the dorsal root ganglion cell is surrounded by the cytoplasm of a satellite cell, there are not four but two laminae in the resulting limiting membrane between the ganglion cell and its supporting cell, although adjoining satellite cell cytoplasm is separated by four lamellae.

Examination of the enfolding of an axon by the Schwann cell has shown very large numbers of partially enclosed fibres (similar to that shown in Pl. 1, fig. 3) in a sort of funnel-shaped depression. It may be that this is a very short part of the fibre as it sinks into or leaves the Schwann cell, or that at an early stage the length of the fibre continues in this sort of relation along the surface of the Schwann cell. The impression gained is that the former is the case, but the question cannot be really elucidated until large numbers of serial sections can be examined. It is, however, of some interest that in the large amount of material that has been examined there has not been a single fibre that could definitely be shown to be without any intimate contact with, or covering by, Schwann cell cytoplasm, emphasizing once more the complete interdependence of the peripheral nerve fibre and the Schwann cell.

SUMMARY

1. The relationship of Schwann cell and nerve fibre in electron micrographs has been examined.
2. The presence of an attachment or mesaxon between the Schwann cell surface and the nerve fibre is confirmed.
3. The detailed relationship between the lamellae in the surface membranes of the Schwann cell, nerve fibre and mesaxon are shown.

We wish to thank Mr S. A. Edwards for technical assistance and the British Empire Cancer Campaign for financial support.

REFERENCES

- CAJAL, S. RAMON Y. (1909). *Histologie du système nerveux de l'homme et des vertébrés*. Paris: Maloine.
- CAUSEY, G. & HOFFMAN, H. (1954). The submicroscopic structure of degenerating and regenerating nerves. *J. Anat., Lond.*, **88**, 554.
- CAUSEY, G. & HOFFMAN, H. (1955). Cytoplasmic synthesis in nerve cells. *Brit. J. Cancer* (in the Press).
- GASSER, H. S. (1952). *Cold Spr. Harb. Symp. quant. Biol.* **17**, 32-36.
- GASSER, H. S. (1955). Properties of dorsal root unmyelinated fibres on the two sides of the ganglion. *J. gen. Physiol.* **38**, 709-728.
- GEREN, B. B. (1954). The formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. *Exp. Cell Res.* **7**, 558-562.
- HESS, A. & LANSING, A. I. (1953). The fine structure of peripheral nerve fibers. *Anat. Rec.* **117**, 175-199.
- NAGEOTTE, J. (1932). *Cytology and Cellular Pathology of the Nervous System*, ed. Penfield, Hoeber, N. Y.
- YOUNG, J. Z. (1942). The functional repair of nervous tissue. *Physiol. Rev.* **22**, 318-374.

EXPLANATION OF PLATES

PLATE 1

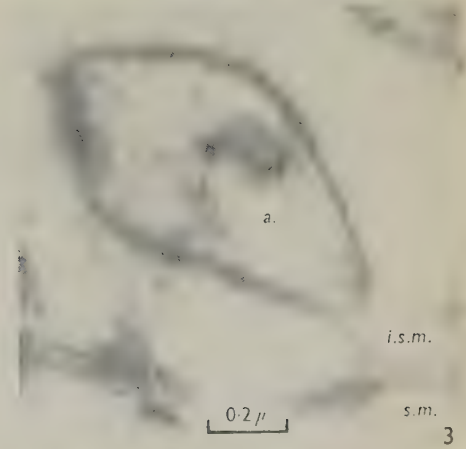
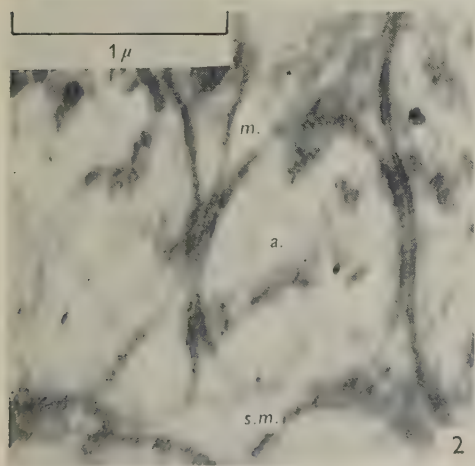
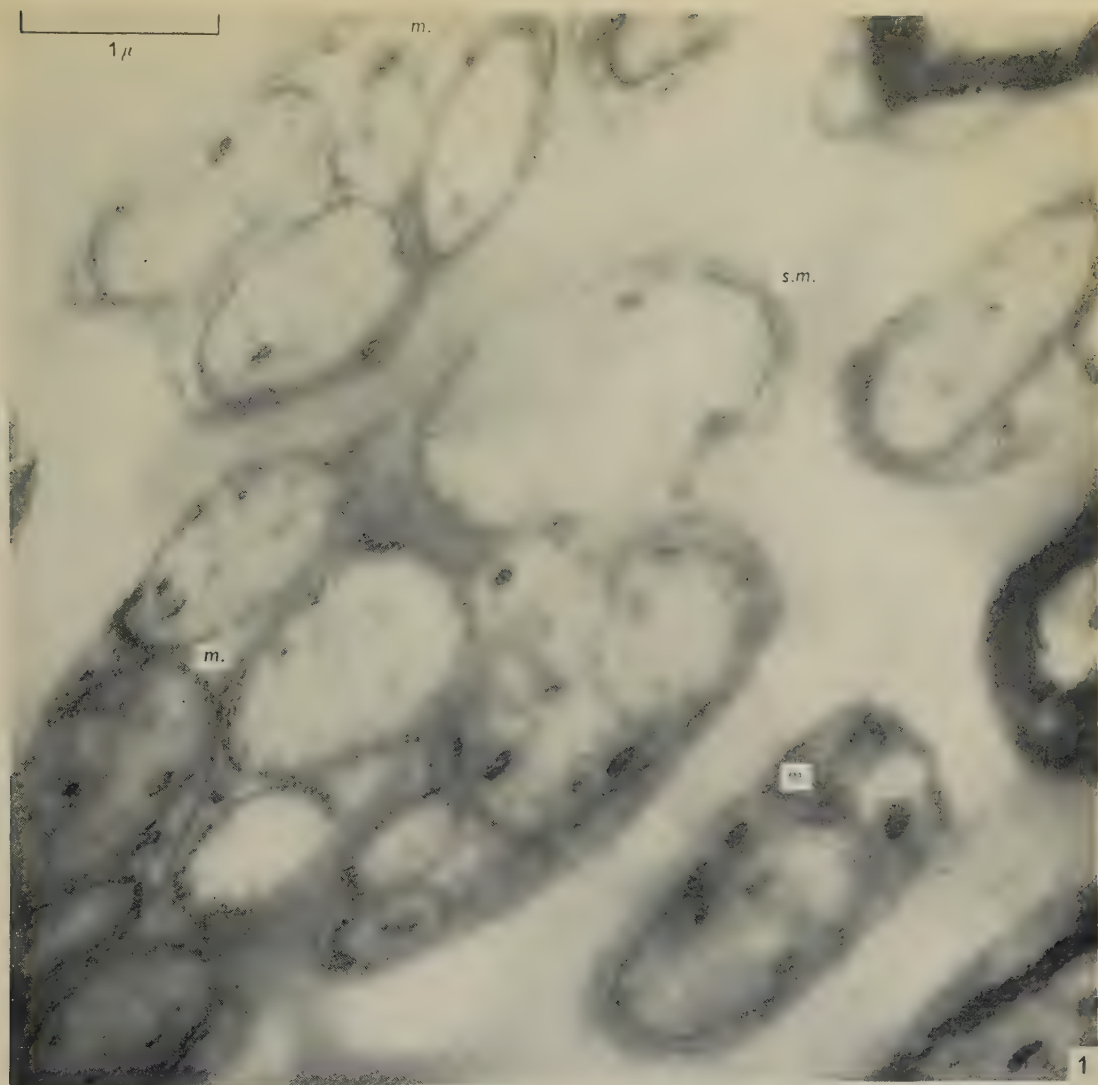
- Fig. 1. Normal vagus nerve of the rat fixed in buffered osmium tetroxide. Two bundles of non-myelinated fibres as well as parts of myelinated fibres and single non-myelinated fibres. *s.m.*, surface membrane of Schwann cell; *m.*, mesaxons.
- Fig. 2. Surface invagination of a Schwann cell by a non-myelinated fibre 'a'. *s.m.*, Schwann membrane; *m.*, mesaxons going to a more deeply placed fibre.
- Fig. 3. Short mesaxon to a non-myelinated fibre. *a.*, axon; *s.m.*, Schwann membrane; *i.s.m.*, inner layer of Schwann membrane forming a mesaxon.

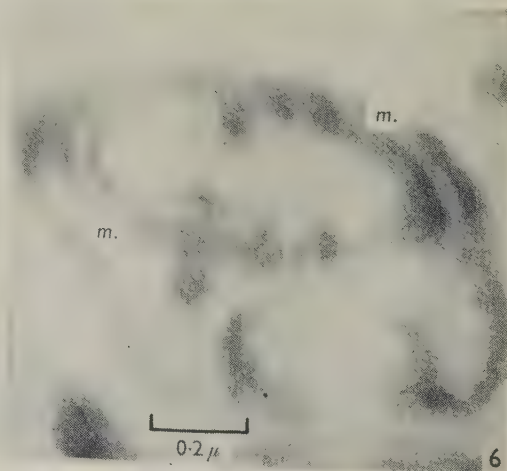
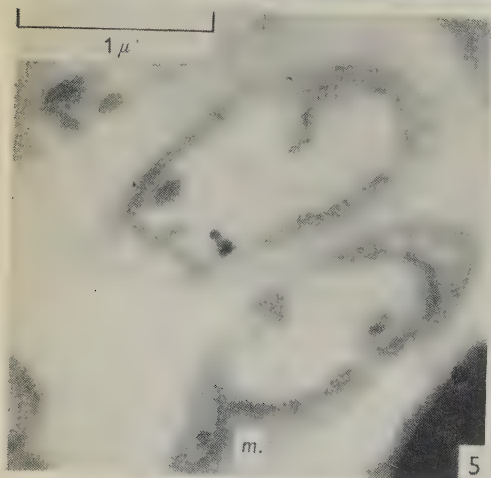
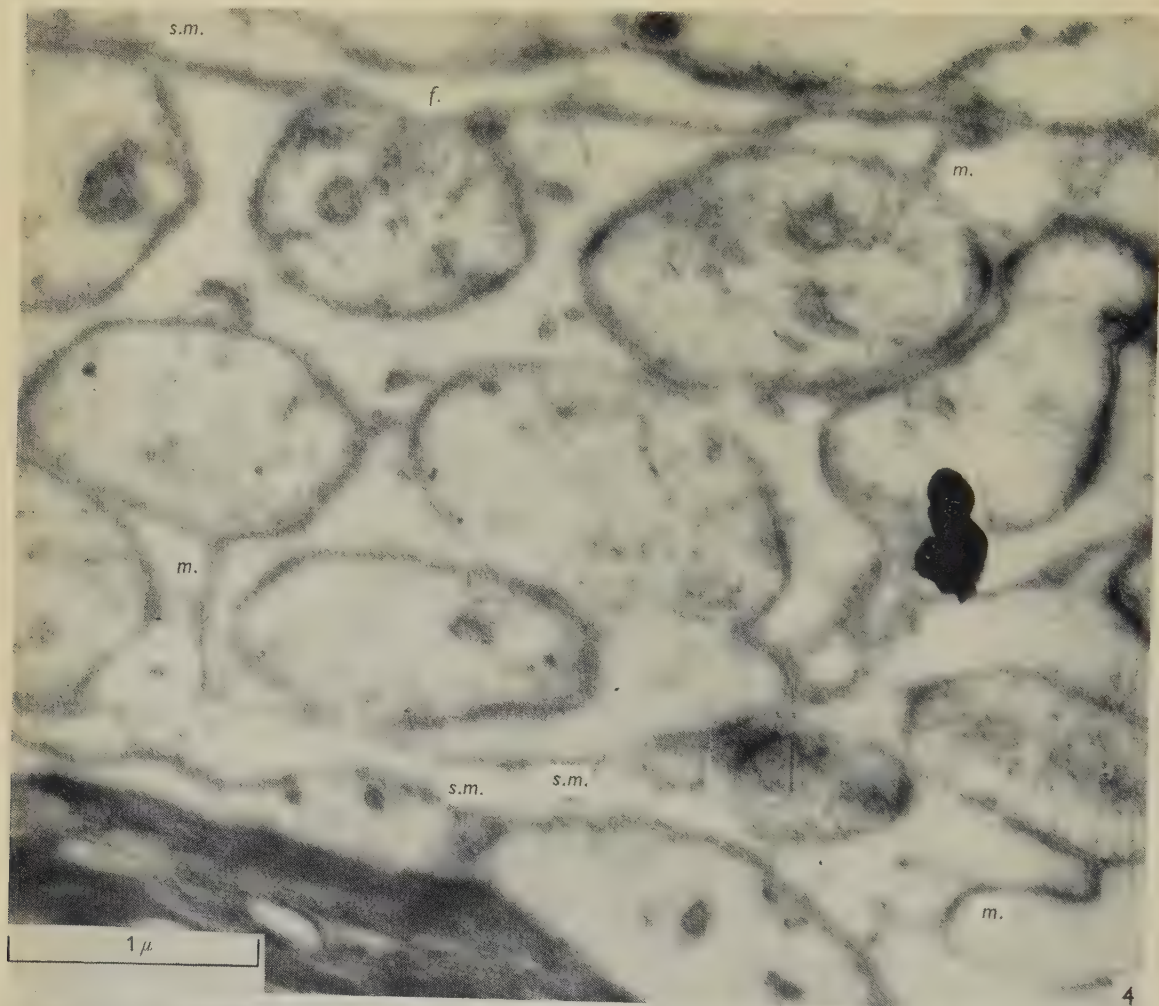
PLATE 2

Fig. 4. Dorsal nerve root of the rat. The edge of a myelinated fibre with surrounding Schwann cell cytoplasm is seen at the bottom left. A bundle of non-myelinated fibres inside the Schwann cell fills the rest of the field. *s.m.*, Schwann membrane; *f.*, funnel-like invagination; *m*, mesaxons.

Fig. 5. Two non-myelinated fibres within Schwann cell cytoplasm. An elongated mesaxon is shown at 'm'.

Fig. 6. Two coils of mesaxon 'm' round an axon within the Schwann cell cytoplasm.





GROWTH CHANGES IN THE DIAMETER OF PERIPHERAL NERVE FIBRES IN FISHES

By P. KYNASTON THOMAS

Department of Anatomy, University College, London

INTRODUCTION

Our understanding of the factors involved in the determination of the diameter of peripheral nerve fibres has been added to considerably during recent years. The importance of contact of the fibres with the periphery has been realized, and has been experimentally analysed in mammalian nerves during regeneration (Weiss, Edds & Cavanaugh, 1945; Sanders & Young, 1945, 1946; Aitken, Sharman & Young, 1947; Aitken, 1949), and during post-natal development (Evans & Vizoso, 1951). Edds (1950) and Fernand & Young (1951) have considered the possible significance of influences acting on the parent cell. Simpson & Young (1945) have investigated the relative importance of the size of the fibres of the proximal stump, and of the size of tubes in the distal stump into which growth occurs, in the determination of the diameter of regenerating fibres during experiments on the cross-union of somatic and visceral nerves. More recently, Cragg (1955) has approached the question by comparing relationships derived from hypotheses concerning the mechanical organization of nerve fibres with the available data as to their structural dimensions obtained by direct measurement.

In this investigation, the changes occurring in the diameter of myelinated fibres in the lateral line nerve of *Salmo trutta* during growth have been examined and considered in relation to the problem of the nature of the factors influencing fibre diameter. This nerve was chosen because of the magnitude of the changes that take place during growth in comparison with the nerves of other vertebrates; diameters of as much as 30μ are found in the largest specimens. The nerve was previously used for studying the growth changes in internodal length and myelin sheath thickness (Thomas & Young, 1949; Thomas, 1955).

METHODS

All measurements were made on the lateral line nerve of the brown trout, *S. trutta*. The selected portions of the nerve were removed, attached to card frames and fixed in Flemming's solution. Paraffin sections (5μ) were stained by a modified Weigert stain (Gutman & Sanders, 1943). Estimates of the diameter of the fibres were made by measurement on photographs taken at a magnification of $\times 750$ by direct projection onto bromide paper. The fibres were divided into 2μ groups by matching with circles of appropriate diameter scored on a thin Perspex square and counted by pricking with a needle activating a mechanical counter. The method has been more fully described by Fernand & Young (1951), who have discussed its accuracy and sources of error.

For the measurements of perineurial diameter, the sections were counterstained with light green and measured directly at a magnification of $\times 100$, using an ocular micrometer. Observations on the time of onset of myelination in young specimens were made on transverse sections of the whole fish, again fixed in Flemming's solution and stained by the Weigert method as above.

RESULTS

(1) *The increase in fibre number and diameter in the lateral line nerve with growth*

In mammals, where there is a limited growth period, fibres become myelinated in peripheral nerves during embryonic life and over a limited portion of the post-natal growth period, the adult number of myelinated fibres being achieved before the cessation of general growth. This has been found by numerous investigators, whose results are summarized by Rexed (1944). Rexed found in man that adult myelinated fibre number was attained in the spinal roots by the age of 9. More recently, Mohiuddin (1951) has reported that in the inferior dental nerve of the cat the full number of axons are present at birth but only about 40% are myelinated. Thereafter the number of myelinated fibres increases until the 8th post-natal week. Similar results have been obtained in amphibians (Hardesty, 1899). No observations of changes in the number of myelinated fibres in fishes, where growth is continuous, have been discovered in the literature.

Myelination begins in the lateral line nerve of *S. trutta* shortly before hatching. Immediately after hatching there are only about ten fibres, with a maximum diameter of 4μ , possessing myelin sheaths in the proximal portion of this nerve. Thereafter, a progressive increase in the number of myelinated fibres at this site occurs throughout the whole size range investigated. The largest specimen it was possible to obtain was 54.5 cm. in length and possessed 1372 myelinated fibres with a maximum diameter of 32μ . Table 1 gives representative counts from eight specimens over this range.

Table 1. *Number of myelinated fibres in lateral line nerve in fishes of different lengths*

Length of fish (cm.)...	1.5	6.4	10.3	15.0	19.8	26.3	37.5	54.5
No. of fibres	10	242	403	469	531	711	871	1372

The precise way in which the diameter of fibres increases during growth has not been examined hitherto, but is of interest in attempting to elucidate the mechanics of nerve growth; the present results have been utilized for this purpose by Cragg (1955). A possible way of investigating this question, and the method that has been adopted here, is to follow the changes in maximum fibre diameter. Fig. 1 shows the results obtained for the proximal portion of the lateral line nerve of *S. trutta*. Maximum diameter rises from an initial value of 4μ to one of 30μ over the size range investigated (fish lengths 1.5–55 cm.). It will be observed that although maximum fibre diameter continues to increase over the whole of this range, the rate of increase is not uniform, being rapid at first and declining progressively throughout growth. The possible significance of the decremental nature of this increase is discussed later.

(2) *Alteration in the distribution of fibre diameters during growth*

Fig. 2 shows representative histograms of frequency-size distributions from five specimens of different lengths for the proximal portion of the lateral line nerve, the values obtained being also set out in Table 2. The nerve from the larger specimens

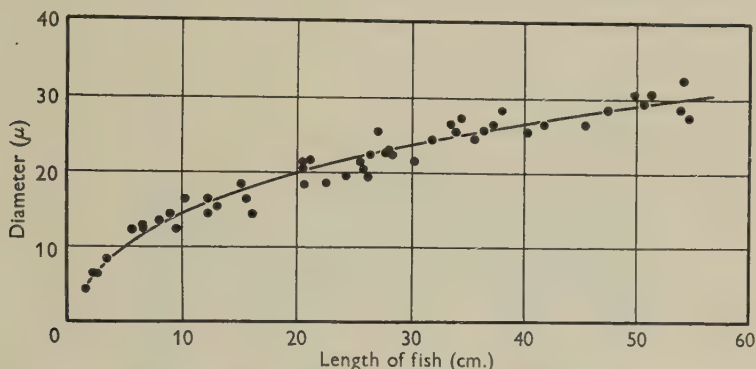


Fig. 1. Lateral line nerve, *S. trutta*. Relationship between maximum fibre diameter and length of fish. Curve drawn by eye.

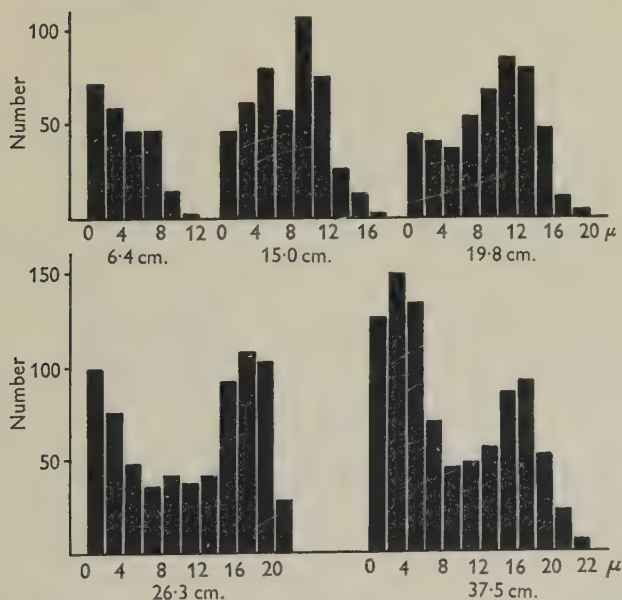


Fig. 2. Lateral line nerve, *S. trutta*. Histograms of frequency-fibre diameter distributions for fishes of different lengths.

shows a bimodal distribution of fibre size. The distribution for the 37.5 cm. fish possesses two groups of fibres with peaks at 2-4 and 16-18 μ and the 26.3 cm. fish shows peaks at the 0-2 and 16-18 μ groups.

In the smaller specimens, however, the nerve does not show a bimodal distribution.

At first there are a relatively greater number of fibres in the smallest diameter groups (6.4 cm. fish), but later the distribution becomes more symmetrical: in the 15 cm. fish the distribution is unimodal with a single peak at 8–10 μ . The skew distribution of the 19.8 cm. specimen provides an intermediate picture.

Table 2. *Change in fibre size distribution with growth*

Length of fish (cm.) ...		6.4	15.0	19.8	26.3	37.5
No. of fibres in diameter groups of 2 μ	0–2	73	47	72	119	126
	2–4	59	62	67	96	150
	4–6	46	80	36	68	133
	6–8	47	55	55	36	69
	8–10	15	108	69	42	46
	10–12	2	76	86	38	48
	12–14	—	27	81	41	56
	14–16	—	13	49	72	86
	16–18	—	1	12	88	92
	18–20	—	—	4	83	53
	20–22	—	—	—	28	12
	22–24	—	—	—	—	6
Total		242	469	531	711	871

The number of fibres in the group with the larger mean diameter remains approximately constant throughout growth and is not added to by fibres becoming myelinated later. This has been the finding of others who have followed the development of the fibre-size distribution pattern of a bimodal nerve: Boughton (1906) examined the oculomotor nerve of the rat, Mohiuddin (1951) the inferior dental nerve of the cat, and Evans & Vizoso (1951) the nerve to the medial head of the gastrocnemius muscle in the rabbit. In other words, the fibres that ultimately become largest are the earliest to become myelinated. This relationship has also been confirmed for the central nervous system by Vogt (1902), Schimert (1941) and Hess (1954). It is also supported by the fact that myelination begins in the lateral line nerve in *S. trutta* appreciably earlier than in the branchial branches of the vagus, the largest fibres of which always remain considerably smaller than those of the lateral line nerve.

The only investigators who have not found this relationship to hold are Kiss & Mihálik (1930), who reported that the sympathetic preganglionic fibres in the ventral roots of man become myelinated before the eventually much larger motor fibres to voluntary muscle. Rexed (1944) also examined the growth of the ventral roots in man and concluded that this observation was erroneous.

The functional significance of the two fibre groups in the lateral line nerve is suggested by the observations of Katsuki, Yoshino & Chen (1951). They have demonstrated that the peripheral part of each organ is innervated by fine myelinated fibres with numerous terminal branches that are distributed to large numbers of cells and which have a low stimulus threshold. The central part of the organ, on the other hand, is innervated by large myelinated fibres which have few terminal branches, that are distributed to a small number of cells and which have a higher stimulus threshold. These differences have led these authors to suggest that the threshold of sensation is determined by the thinner fibres and that the larger fibres are concerned with precise discrimination.

(3) Variation in the distribution of fibre diameters along the length of the lateral line nerve

Sections were examined at intervals along the length of the lateral line nerve from its point of separation from the visceral branch of the vagus in two specimens, 25.5 and 26.3 cm in length. The changes found were similar in both nerves and are shown for the latter nerve in Table 3, and also in the histograms of percentage frequency-size distributions of Fig. 3. The nerve is strongly bimodal, and it will be

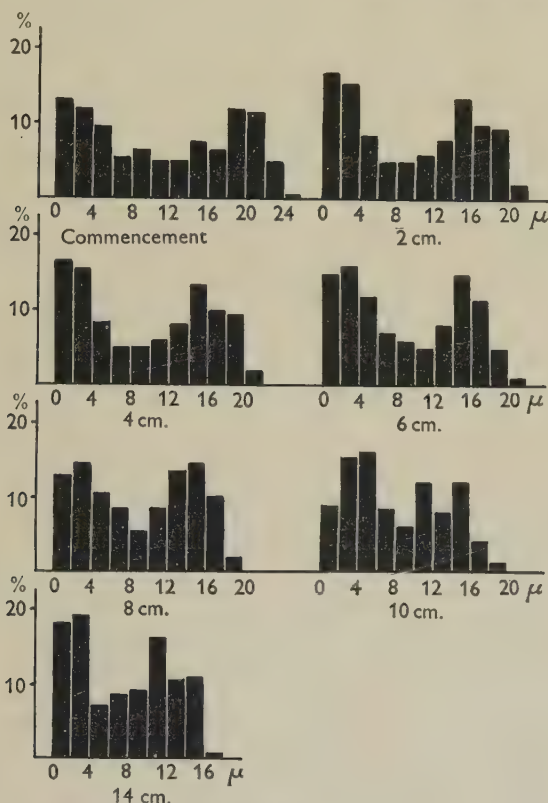


Fig. 3. Lateral line nerve, *S. trutta* (length 26.3 cm.). Histograms of percentage frequency-fibre diameter distributions at successive intervals along the nerve.

observed that both fibre groups persist throughout the length of the nerve and that the relative proportions of fibres in each do not alter noticeably. The most evident change in the pattern of fibre size distribution is the reduction of the modal value of the group with the larger mean diameter with a consequent obscuring of the trough between the two groups. This has been noticed by other observers, for example by Nisimoto (1939) in the nerves to the hindlimb of the cat, and attributed either to tapering or to dichotomy of fibres or to a combination of these factors. The branches of a dichotomizing fibre are generally of smaller diameter than the parent fibre, although their combined areas exceed that of the parent fibre. A differential

loss of larger fibres to the more proximal parts of the lateral line canal would not produce this progressive shift of the upper mode.

The existence of a true conical diminution in fibre size has been disputed. Rexed (1944), examining the phrenic nerve, and Causey (1948), the nerve to the medial head of the gastrocnemius muscle of the rabbit, could find no evidence of the tapering of fibres; but Dunn (1902), studying the nerves to the hindlimb of the frog, and Fernand & Young (1951), the same nerves in the rabbit, considered that tapering probably did occur in these nerves.

Since over most of the lateral line nerve fibres constantly leave to supply the lateral line organs, it is difficult to be certain here whether either tapering or dichotomy or both of these factors in combination are responsible for the observed reduction in fibre size. However, the first 4 cm. of the nerve on which the measurements given in Table 3 and Fig. 3 were made, represent the segment of the nerve after its point of separation from the visceral branch of the vagus, but before it came to underlie the lateral line canal. Over this stretch there is a relatively small change in the number of fibres in the nerve, the total number falling from 749 to 682. Yet a reduction in the value of the upper mode is clearly evident. This suggests that the diminution in the calibre of the fibres occurs in the absence of branching. It is likely that this is also true for the rest of the nerve for no branching of fibres was observed during the isolation of single fibres from this part of the nerve for inter-nodal length measurement in a considerable number of specimens.

Table 3. *Variation in fibre size distribution along the lateral line nerve*

Distance along nerve (cm.) ...		0	2	4	6	8	10	14
No. of fibres in diameter groups of 2μ	0-2	99	119	112	94	59	30	27
	2-4	91	96	105	97	64	55	29
	4-6	73	68	57	76	48	56	11
	6-8	43	36	34	43	38	31	13
	8-10	48	42	32	37	26	21	14
	10-12	38	38	42	32	39	42	24
	12-14	38	41	56	52	61	27	16
	14-16	55	72	92	94	66	42	17
	16-18	48	88	70	72	47	27	1
	18-20	92	83	67	32	9	14	—
	20-22	85	28	15	5	—	3	—
	22-24	37	—	—	—	—	—	—
	24-26	2	—	—	—	—	—	—
Total		749	711	682	634	457	348	152

Although there is this reduction in fibre size along the length of the nerve, no definite indication of a differential distribution of fibres with respect to fibre size to the organs along the lateral line canal is evident. As just observed, both groups of fibres persist throughout the length of the nerve and the relative proportions of the fibres in each do not alter greatly. It is thus clear that there is no relationship detectable in this nerve between the length and the diameter of the fibres. The possibility of such a relationship was first suggested by Schwalbe (1882), who claimed for the spinal nerves of the cat that longer fibres have larger diameters than fibres of shorter length, but his grounds for making this claim were later criticized by Dunn (1902) who found the opposite relationship to obtain in the hindlimb of the

frog. Similarly, Lloyd & Chang (1948) reported that in the cat the fibres to the crural muscles are of larger diameter than those to the more distal femoral group, and Fernand & Young (1951) demonstrated that the nerves innervating the distal musculature in the hindlimb of the rabbit contain smaller fibres than those innervating the more proximal muscles.

Although the explanation of these findings in the hindlimbs of the frog, cat and rabbit may partly include the effect of branching and tapering of fibres, the important determining factor, as stressed by Fernand & Young, is the level of origin of the fibres in the spinal cord. The fibres of largest diameter in the peripheral nervous system are those that arise from the regions of the cord where differentiation occurs earliest, namely the cervical and lumbar dilatations. These authors have made an interesting suggestion to account for the larger diameter of the fibres innervating the proximal limb muscles. They postulate that the earliest fibres to differentiate and reach the limb buds innervate the more proximal muscles and are therefore subjected to the influence of contact with the end-organ for longer than later differentiating fibres innervating the more peripheral muscles. This is not supported, however, by the findings in the lateral line nerve. Here the more proximal organs of the lateral line canal are not innervated by larger fibres than the more distal, apart from the reduction in fibre diameter occurring along the length of the nerve most probably attributable to tapering.

CONCLUSIONS

The determination of fibre diameter

The present investigation has emphasized the fact that there exists a definite relationship between the time at which a fibre acquires its myelin sheath and the diameter it finally attains. The observation that the largest fibres in the spinal nerves in mammals are found in the roots arising from the cervical and lumbar enlargements, where neural differentiation begins in the cord, suggests that it may be possible for this relationship to be pressed further back into ontogeny and that the ultimate diameter of a fibre depends upon the time of differentiation of its parent cell.

Thus in the lateral line nerve of *S. trutta*, a fibre that has reached the critical diameter at which it becomes myelinated, continues to increase in diameter throughout the life of the animal, its diameter at any stage depending upon the time that has elapsed since it became myelinated. Fibres becoming myelinated later do not attain the diameter of their predecessors.

The increase in the diameter of a fibre, however, does not keep pace with its longitudinal growth as evidenced by the change in the distance between the nodes of Ranvier (Thomas, 1955), nor does it keep pace with the increase in the diameter of the nerve trunk. Measurements made on *S. trutta* (unpublished) have shown that the growth of the latter is isometric, the perineurial diameter remaining proportional to the length of the trunk throughout growth. The significance of the decremental nature of the increase of fibre diameter is not yet clear, although Cragg (1955) has suggested that it can be related to the restrictive action of the myelin and connective tissue sheaths surrounding the axon, which limit its growth. The concept of

the organization of peripheral myelinated fibres developed by Young (1945) implies that the neurilemma exercises a mechanical restrictive action, the myelin layer being compressed against it by the axon. The contents of the perineurium are also known to be under restraint since the making of a slit in this structure results in the bulging outwards of the contained fibres through the gap (Sunderland, 1946), and its complete removal produces considerable swelling of the nerve (Shanes, 1952). The present observations are consistent with Cragg's suggestion, good agreement existing between the measurements of the change in maximum fibre diameter during growth and those expected on theoretical grounds. Alternatively, these changes may reflect an intrinsic difference between the rate of growth of fibre diameter and that of the skeletal parts upon which internodal length is dependent (see Thomas, 1955).

Although such a mechanism may be responsible for the general determination of fibre diameter during development, it is clear that fibre size is affected by various additional factors operating during growth and at maturity. The influence of peripheral factors is important. This has been demonstrated experimentally both for regenerating fibres (Weiss *et al.* 1945; Sanders & Young, 1945, 1946; and others), and during post-natal development (Evans & Vizoso, 1951), and a naturally occurring instance has been provided by Herrick (1902). He found in fishes that occasional lateral line organs of reduced size are encountered, and that the fibres innervating them have smaller diameters than those innervating normal organs. There are also indications that centrally acting influences are of significance. Thus, it has been suggested that a reduction in the diameter of a fibre may follow a diminution in the afferent stimulation to the parent cell (Detwiler & Lewis, 1925), this perhaps being comparable, in lesser degree, to the phenomenon of trans-neuronal degeneration, and Edds (1950) has postulated that the increase in diameter of fibres to functionally overloaded muscles may result from augmented stimulation of the parent motor neurons by an increased inflow of proprioceptive impulses.

SUMMARY

1. The number of myelinated fibres in the lateral line nerve of *Salmo trutta* increases continuously throughout the life of the animal.
2. The diameter of the largest fibres in the nerve increases progressively with increasing fish length, but the rate of increase, which is rapid at first, progressively declines. The possible significance of this finding is discussed.
3. The distribution of fibre diameters in the nerve alters during growth, being at first unimodal and later bimodal. A relationship exists between fibre diameter and time of myelination; the larger fibres in the nerve acquire myelin sheaths before the smaller.
4. The variation in the distribution of fibre diameters along the length of the nerve is considered. No relationship between the length and diameter of fibres was detected apart from changes that suggest the occurrence of tapering of fibres.

I wish to thank Prof. J. Z. Young for his advice and criticism and Mr R. H. Ansell for technical assistance.

REFERENCES

- AITKEN, J. T. (1949). The effects of peripheral connexions on the maturation of regenerating fibres. *J. Anat., Lond.*, **83**, 32-43.
- AITKEN, J. T., SHARMAN, M. & YOUNG, J. Z. (1947). Maturation of regenerating nerve fibres with various peripheral connexions. *J. Anat., Lond.*, **81**, 1-22.
- BOUGHTON, T. H. (1906). The increase in number and size of the medullated fibers in the oculo-motor nerve of the white rat and of the cat at different ages. *J. comp. Neurol.* **16**, 153-165.
- CAUSEY, G. (1948). The effect of pressure on nerve fibres. *J. Anat., Lond.*, **82**, 262-270.
- CRAGG, B. G. (1955). A physical theory of the growth of axons. *J. cell. comp. Physiol.* **45**, 33-60.
- DETWILER, S. R. & LEWIS, R. W. (1925). Size changes in primary brachial motor neurones following limb excisions in ablystoma embryos. *J. comp. Neurol.* **39**, 291-300.
- DUNN, E. H. (1902). On the number and on the relation between diameter and distribution of the nerve fibers innervating the leg of the frog, *Rana viriscens brachicephalica* Cope. *J. comp. Neurol.* **12**, 291-328.
- EDDS, MACV. (1950). Hypertrophy of nerve fibers to functionally overloaded muscles. *J. comp. Neurol.* **93**, 258-276.
- EVANS, D. H. L. & VIZOSO, A. D. (1951). Observations on the mode of growth of motor nerve fibers in rabbits during post-natal development. *J. comp. Neurol.* **95**, 429-461.
- FERNAND, V. S. V. & YOUNG, J. Z. (1951). The sizes of the nerve fibres of muscle nerves. *Proc. Roy. Soc. B* **139**, 38-58.
- GUTMAN, E. & SANDERS, F. K. (1943). Recovery of fibre numbers and diameters in the regeneration of peripheral nerve. *J. Physiol.* **101**, 489-518.
- HARDESTY, I. (1899). The number and arrangement of the fibers forming the spinal nerves of the frog (*Rana viriscens*). *J. comp. Neurol.* **9**, 64-112.
- HERRICK, J. (1902). A note on the significance of the size of nerve fibers in fishes. *J. comp. Neurol.* **12**, 329-334.
- HESS, A. (1954). Post-natal development and maturation of the nerve fibers of the central nervous system. *J. comp. Neurol.* **100**, 461-480.
- KATSUKI, Y., YOSHINO, S. & CHEN, J. (1951). Neural mechanism of the lateral line organ of fish. *Jap. J. Physiol.* **1**, 264-267.
- KISS, F. & MIHÁLIK, P. (1930). Über die Markreifung im peripherischen Nervensystems. *Anat. Anz.* **69**, 433-444.
- LLOYD, D. P. C. & CHANG, H. T. (1948). Afferent fibers in muscle nerves. *J. Neurophysiol.* **11**, 199-207.
- MOHIUDDIN, A. J. (1951). The postnatal development of the inferior dental nerve of the cat. *J. Anat., Lond.*, **85**, 24-35.
- NISIMOTO, K. (1939). Über die kaliber- und Zahlenschwankung der markhaltigen Nervenfasern im Verlauf der peripheren Nervenäste. *Jap. J. med. Sci. (Anat.)*, **7**, 373-383.
- REXED, B. (1944). Contributions to the knowledge of the post-natal development of the peripheral nervous system in man. *Acta Psychiat. (Suppl.)*, no. 33.
- SANDERS, F. K. & YOUNG, J. Z. (1945). Effect of peripheral connexion on the diameter of nerve fibres. *Nature, Lond.*, **155**, 237-238.
- SANDERS, F. K. & YOUNG, J. Z. (1946). The influence of peripheral connexion on the diameter of regenerating nerve fibres. *J. exp. Biol.* **22**, 203-212.
- SCHIMMERT, J. S. (1941). Die Bedeutung des Faserkalibers und der Markscheidendicke im Zentralnervensystem. *Z. ges. Anat. I. Z. Anat. EntwGesch.* **111**, 201-225.
- SCHWALBE, G. (1882). *Über die Kaliberverhältnisse der Nervenfasern*. Leipzig: Vogel.
- SHANES, A. (1952). Effects of sheath removal on bullfrog nerve. *J. cell. comp. Physiol.* **41**, 305-311.
- SIMPSON, S. A. & YOUNG, J. Z. (1945). Regeneration of fibre diameter after cross-unions of visceral and somatic nerves. *J. Anat., Lond.*, **79**, 48-65.
- SUNDERLAND, S. (1946). The effect of rupture of the perineurium on the contained fibres. *Brain*, **69**, 149-152.
- THOMAS, P. K. (1955). Growth changes in the myelin sheath of peripheral nerve fibres in fishes. *Proc. Roy. Soc. B*, **143**, 380-391.

- THOMAS, P. K. & YOUNG, J. Z. (1949). Internode lengths in the nerves of fishes. *J. Anat., Lond.*, **83**, 336-350.
- VOGT, O. (1902). Der Wert der myelogenetischen Felder der Grosshirnrinde (Cortex pallii). *Anat. Anz.* **29**, 273-287.
- WEISS, P., EDDS, MACV. & CAVANAUGH, M. (1945). The effect of terminal connexions on the caliber of nerve fibers. *Anat. Rec.* **92**, 215-233.
- YOUNG, J. Z. (1945). History of the shape of a nerve fibre. (From *Essays on Growth and Form*, presented to D'Arcy Wentworth Thompson.) Oxford: Clarendon Press.

AN EXPERIMENTAL STUDY OF THE SECONDARY OLFACTORY CONNEXIONS IN *TESTUDO GRAECA*

By H. J. GAMBLE

*Department of Anatomy, St Mary's Hospital Medical School,
Paddington, London, W. 2*

INTRODUCTION

Secondary olfactory connexions have received much attention in recent years, and experimental studies have shown that they are less extensive in mammals than had been thought (Clark & Meyer, 1947; Meyer & Allison, 1949). Experimental investigations of this problem in one reptilian species (*Lacerta viridis*—Goldby, 1937; Gamble, 1952*a*) have indicated that here also secondary olfactory connexions are of very much more limited extent than commonly been supposed. It has been shown, moreover (Gamble, 1952*a*) that secondary olfactory fibres in *Lacerta* cross via the habenular commissure to the opposite hemisphere, and that the anterior commissure in this species is in no way concerned with the secondary olfactory pathways. It seemed desirable that these findings, the one so similar to, the other so markedly different from, the findings in mammals, should be investigated in another and less specialized reptilian species, and for this *Testudo graeca* was chosen.

Despite such obvious specialization as the carapace and plastron, the Chelonia are the only living reptiles which have preserved a skull form at all closely resembling that of the primitive anapsid reptiles from which all later amniotes have been derived. It might reasonably be expected that the Chelonian brain would show features characteristic of early reptiles in general; certainly the published descriptions of Chelonian brains (Johnston, 1915; Schepers, 1948) suggest that specialization is little advanced.

MATERIALS AND METHODS

Specimens of *T. graeca* of both sexes and averaging about 6 inches in length were used. Both before and after operation the animals were kept in a cage heated to approximately 30° C.

Operative technique

Ether being apparently valueless, chloroform anaesthesia was used, and the addition of one to two drops of ammonia, apparently acting as a respiratory stimulant, facilitated the onset of anaesthesia to a sufficient depth. The skull was opened with a dental drill and the dura incised. Lesions in the brain were made with a cystotome or small curette. The wound was then filled with penicillin and sulphathiazol crystals and sealed with a solution of Perspex in acetone which dried rapidly to form a hard covering.

The lesions consisted in the removal of, or damage to, the main olfactory bulb, or the main and accessory olfactory bulbs, both unilaterally and bilaterally. Damage

to the accessory olfactory bulb alone was also attempted. The extreme shortness of the olfactory peduncle, and the situation of the accessory bulb (inset, as it were, into the dorsum of the main bulb), made lesions precisely limited to one or other bulb virtually impossible. Mortality was low (1 in 12) and, when it occurred, death appeared to be due to subdural bleeding. In none of the animals was any change in behaviour noted; feeding was often resumed within an hour of consciousness being regained.

Histological methods

(i) Nissl series were prepared from normal specimens. The brains were fixed in formol-acetic-alcohol and complete series were cut in the three primary planes after paraffin embedding. The sections were stained with cresyl violet or with a mixture of cresyl violet and thionone.

(ii) Silver impregnated series were prepared by Nonidez's (1939) technique, and sections cut at 7.5 and 10 μ . The normal series consisted of one in a transverse and one in a horizontal plane. The experimental material consisted of eleven brains from which satisfactory serial sections were obtained and in which the lesion, survival time, etc., were as follows:

(a) Unilateral damage to or removal of main olfactory bulb: one specimen, surviving 8 days.

(b) Unilateral damage to or removal of main and accessory olfactory bulbs: seven specimens, surviving 5, 6, 7, 9, 10, 11 days.

(c) Unilateral damage to accessory olfactory bulb: one specimen surviving 7 days.

(d) Bilateral damage to, or removal of, main and accessory olfactory bulbs: three specimens, two surviving 10 days, the other for 8 days.

The olfactory and related systems in normal material

Certain features of the brain of *T. graeca* were described briefly by Goldy (1936); much longer accounts of the brain of other Chelonians are also available, notably those by Johnston (1915, 1923) for *Cistudo carolina* and by Schepers (1948) for *Testudo geometrica*. From these accounts it appears that both *Cistudo carolina* and *Testudo geometrica* resemble *T. graeca* very closely in cerebral morphology. Johnston and Schepers, however, were largely concerned with evolutionary problems and with the institution of homologies, often of a very detailed and somewhat speculative character, which are not relevant to the present investigation. It seemed desirable, therefore, to summarize the main features of the Chelonian forebrain, as seen in *T. graeca*, with particular emphasis on those parts most directly concerned with olfaction.

The olfactory bulb is relatively very large and is attached to the hemisphere by a short thick peduncle as in primitive mammals; the accessory bulb is small and is situated on the dorsal and posterior aspect of the main bulb. The hemisphere is elongated, its posterior pole overlapping the anterior part of the midbrain, and is marked by two longitudinal fissures: a dorso-lateral fissure in the anterior half or two-thirds of the hemisphere which, because of its relation to underlying cortical areas, has been homologized with the rhinal fissure of mammals, and a ventro-lateral fissure. The latter, as it is followed caudally, veers towards the basal surface

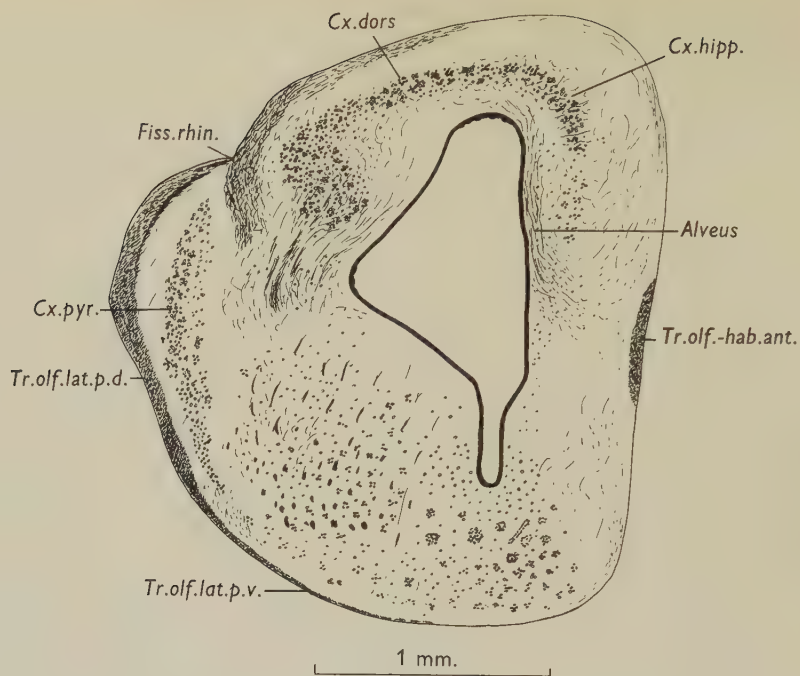
of the hemisphere, and again because of its relationship to underlying structures has been identified as the endorhinal fissure anteriorly and the amygdaloid fissure posteriorly (Johnston, 1915).

The cell masses of the hemisphere can be described briefly as follows. An ill-defined cellular zone surrounding the root of the olfactory peduncle forms the anterior olfactory nucleus, very detailed accounts of which were given by Crosby & Humphrey (1939) for several chelonians. The general arrangement in all is similar to that seen in *Testudo*. Behind this nucleus the hemisphere may be divided into basal and pallial (dorsal) regions. The pallial region contains the cortical areas of which there are three, hippocampal, dorsal and pyriform, arranged as longitudinal strips, situated medially, dorsally and laterally respectively. The basal region consists of the paraterminal body or septum medially and a ventro-lateral cell mass commonly referred to as the 'strio-amygdaloid' complex. It will be noted that this terminology, as well as that of the cortical areas, is largely based on generally accepted mammalian homologies; it should be noted also that the lateral boundary between pallial and basal structures is not very definite, since the ventro-lateral edge of the pyriform cortical area encroaches on the region occupied by the strio-amygdaloid complex.

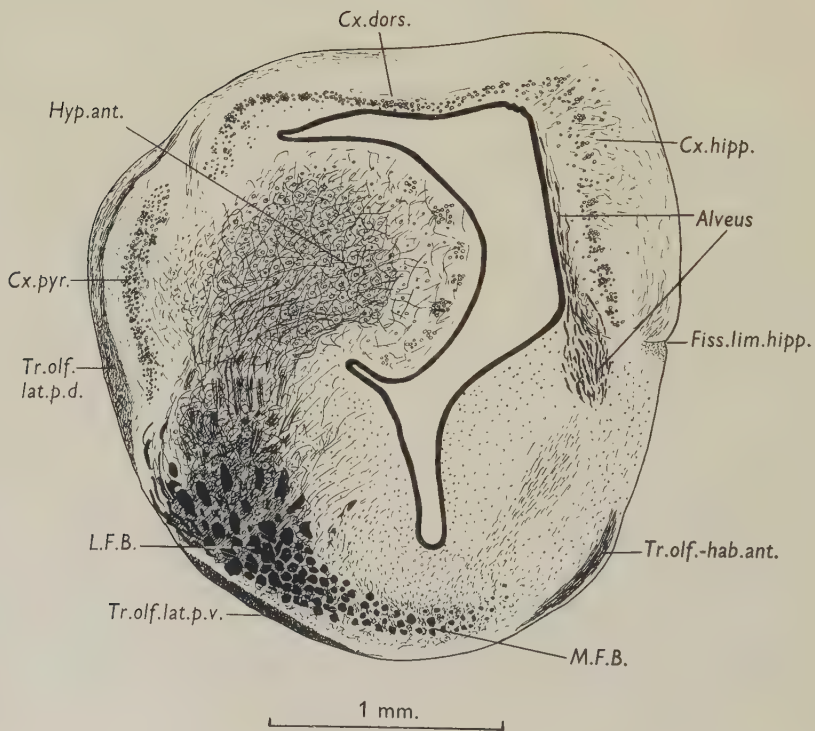
The cortical area chiefly relevant to this investigation is the pyriform. It consists of a cell-lamina in which many of the cells are fusiform in shape and arranged perpendicular to the surface, though many other cells, less easily classified, are present. It can be divided into a dorsal part where the cells are large and compactly arranged and a ventral part where they are smaller and more scattered (Text-figs. 1, 2). Posteriorly this ventral part forms the nucleus of the lateral olfactory tract, and becomes continuous with certain basal structures to be described later.

Little need be said here of the other two cortical areas. Both are poorly differentiated in comparison with many other reptiles, and some of their cytoarchitectural features can be seen in Text-figs. 1-6. The hippocampal area shows the usual division into large- and small-celled parts (Text-fig. 4), although neither is very extensive or clearly defined. The contrast with such reptiles as *Anolis* and the geckos among Lacertilia and with many snakes is very marked. The dorsal cortical area is the least well defined, and its cells for the most part remain in the primitive position close to the ependyma (Text-figs. 2-5); laterally it is separated from the pyriform cortex over most of its extent by a cell-free gap, which coincides with the overlying rhinal fissure. Medially it is continuous with the large-celled part of the hippocampus, but differences in cell size and arrangement enable a distinction to be made. Anteriorly the three cortical areas all blend with the anterior olfactory nucleus, posteriorly with an undifferentiated sheet of cells covering the posterior pole.

Of the basal structures the paraterminal body or septum forms the whole thickness of the medial wall of the hemisphere below the hippocampal cortex and anterior to the interventricular foramen. It is comparatively small and shows only very indistinct nuclear differentiation in the tortoise; its connexions are thought to be largely with the hippocampal cortex, the hypothalamus and the olfactory tubercle, but so far as the present work is concerned the most significant bundle of fibres related to it is the so-called 'anterior olfacto-habenular tract' which is described below.

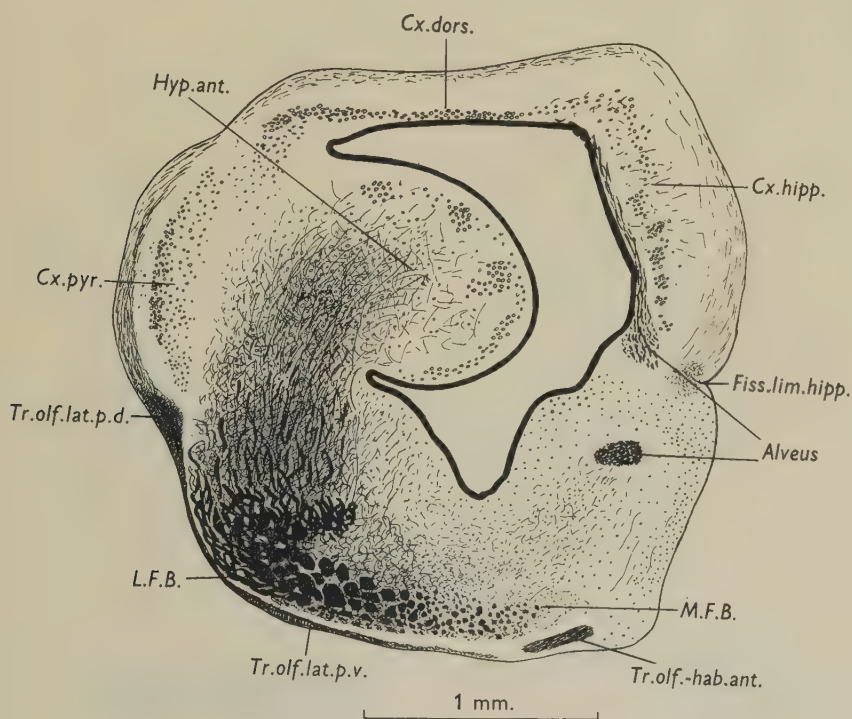


Text-fig. 1



Text-fig. 2

The main element in the basal structures of the hemisphere is the laterally situated strio-amygdaloid complex. On the basis of cell type and distribution three components can be recognized in it. A collection of mainly small and clumped cells extends from the anterior olfactory nucleus to the level of the lamina terminalis; it is bounded dorsally by a deep ventricular sulcus and extends medially round the ventral angle of the ventricle into relation with the paraterminal body (Text-figs. 1, 2). This is the palaeostriatum, or 'olfacto-striatum' as it has sometimes been

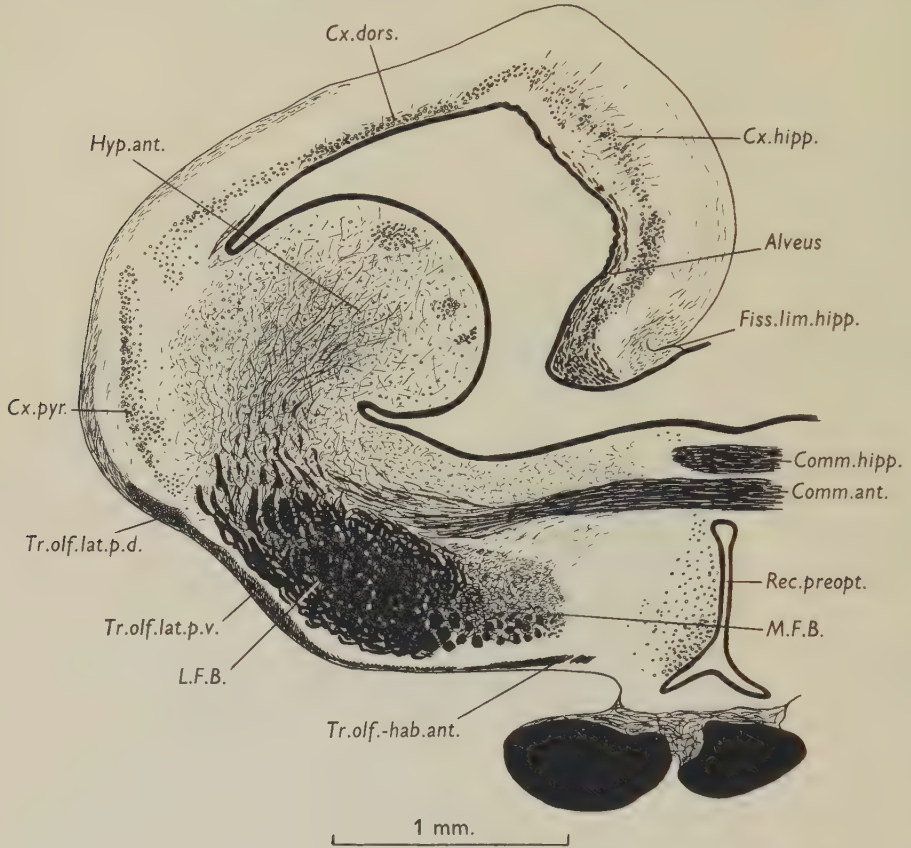


Text-fig. 3

called. Ventrally it bulges slightly from the surface and is covered by a very ill-defined layer of cells, the 'olfactory tubercle' of common usage. The whole palaeostriatum is permeated by the basal forebrain bundle system of fibres, and like this system, may be divided, somewhat arbitrarily, into medial and lateral parts.

A massive projection into the ventricle dorsal to the palaeostriatum (Text-figs. 2-6) extends throughout the length of the ventro-lateral cell mass. Its cells form a thick lamina close to the ependyma and tend to be arranged in clumps. Posteriorly these cells are in apparent continuity with those of the pyriform cortex (Text-fig. 5), but anteriorly there is continuity with neither pyriform nor dorsal cortices. The concavity of the main cellular lamina contains smaller, scattered cells. The whole structure is known as the hypopallium and can be subdivided, on the basis of its appearance in silver impregnated sections, into anterior and posterior

parts. The anterior part contains a dense plexus of fibres which fan out beneath the cellular lamina and appear to join the the lateral forebrain bundle (Text-fig. 2). Posteriorly these connexions are lacking, and instead bundles of fibres are found lying next to the ependyma and apparently running to or from the anterior commissure. Some of these fibres reach laterally to the deep surfaces of the dorsal and pyriform cortices (Text-figs. 5, 6).

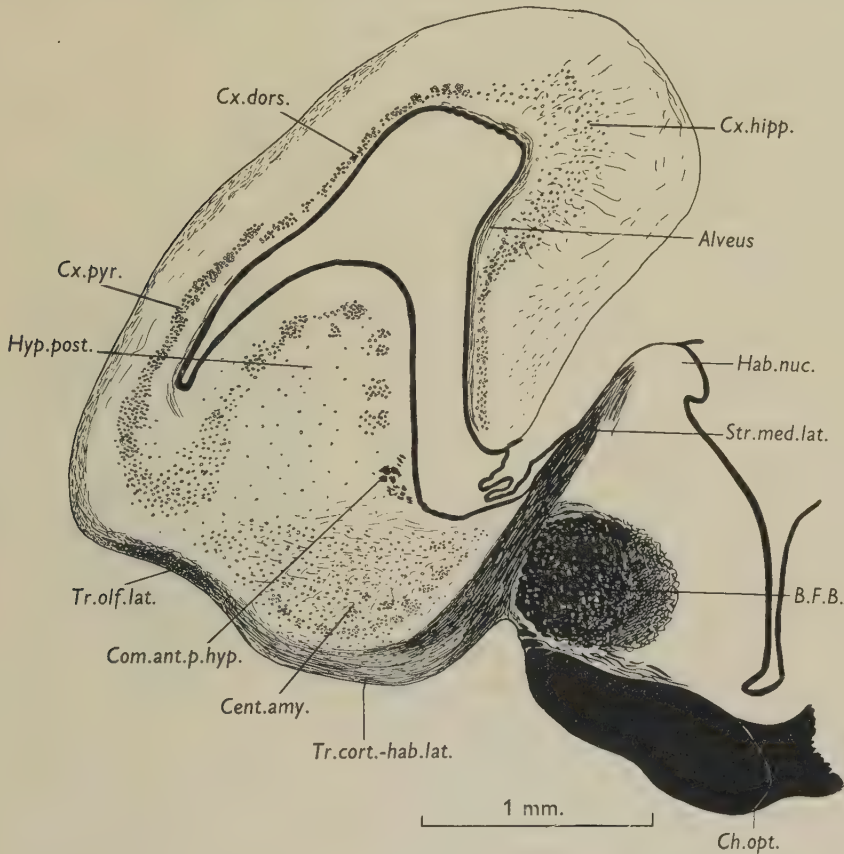


Text-fig. 4

Behind the palaeostriatum, and below the posterior part of the hypopallium, lies a large but rather ill-defined collection of cells, which has a tenuous continuity with the nucleus of the lateral olfactory tract. Fibres of the lateral olfactory tract lie on its surface where they appear to end, while its deeper cells are reached by or give origin to fibres of the anterior commissure and stria terminalis. By its situation and apparent connexions this structure exhibits the major characteristics of an amygdaloid nucleus, and is referred to hereafter as the central amygdaloid mass.

Of the fibre tracts only those relevant to the present investigation will be described. The most important of these are the secondary olfactory fibres which stream posteriorly from between the mitral and granule cell layers of the bulb. It is not possible to distinguish main from accessory bulbar fibres. All these fibres collect

ventro-laterally in the peduncle, and after passing the anterior olfactory nucleus they spread to form a broad layer over the hemisphere extending from the rhinal fissure above to the olfactory tubercle below (Text-fig. 1). Where they cover the anterior part of the pyriform cortex and the lateral part of the olfactory tubercle they are arranged in a plexiform manner, running in all directions, suggestive of

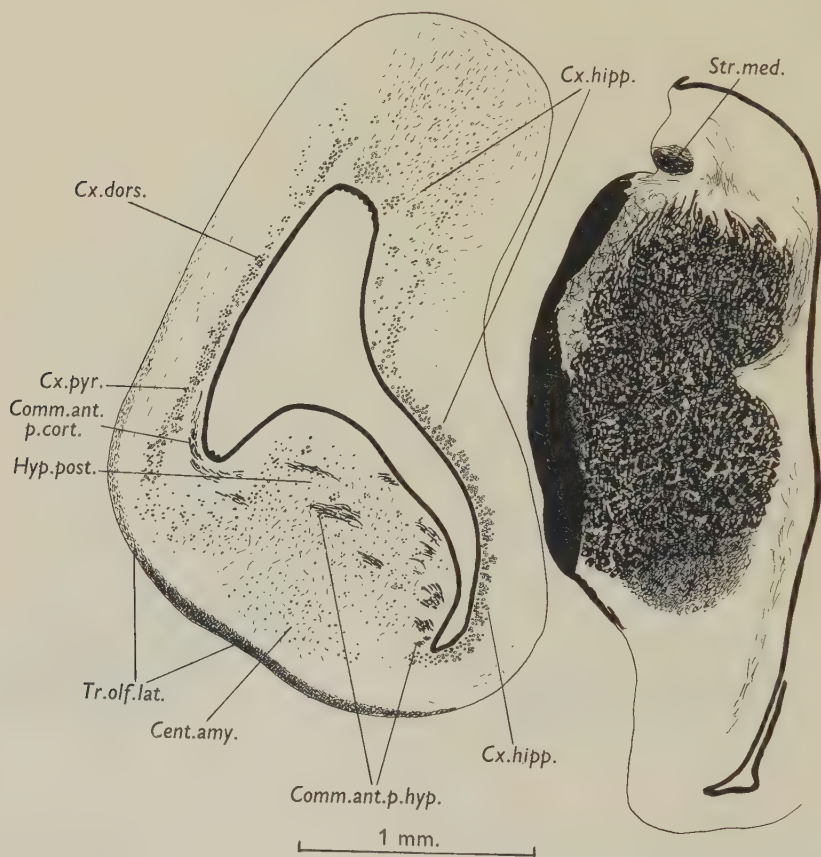


Text-fig. 5

connexions being effected with the underlying cells. A little posterior to this level (Text-fig. 2) fibres of the lateral forebrain bundle reach the surface, and the stream of secondary olfactory fibres is separated into dorsal and ventral parts for a short distance, but these parts soon come together (Text-fig. 4) so that the broad continuity of the tract is regained. At this and at more posterior levels only a few fibres are found superficial to the cells of the pyriform cortex, most being aggregated on the surface of the nucleus of the lateral olfactory tract. With this nucleus they pass to the central amygdaloid mass (Text-figs. 5, 6).

The fibres so far described form the lateral olfactory tract, which is particularly large in *Chelonia*. So far as normal material is concerned it is impossible of course to say whether the more posterior fibres have come directly from the mitral cells of the

bulb, or whether there have been intermediate relays, for example, in the anterior olfactory nucleus. The more ventrally situated fibres which spread on to the olfactory tubercle probably represent the medial olfactory tract described by Johnston (1915). According to Johnston, some of these fibres extended even more medially into the paraterminal (his paraolfactory) region and to the hippocampus. This did not appear to be the case in *Testudo graeca*, and one may add also that nothing was found resembling the so-called olfactory component of the anterior commissure present in many other reptiles.



Text-fig. 6

Experimental degenerations in *Lacerta viridis* (Gamble, 1952a) have shown that a considerable part of a system of fibres known as the stria medullaris consisted of secondary olfactory fibres. In *Testudo* the stria medullaris is a composite bundle on the anterior and dorsal aspects of the thalamus (Text-fig. 6), extending from the habenular commissure (where most of its fibres cross) into the basal part of the hemisphere below the interventricular foramen (Text-fig. 5). In this region the stria is joined by three sets of fibres. From the lateral side it is joined by fibres which appear to stream medially from two sources: (i) from the ventral part of the lateral

olfactory tract (Text-fig. 5), and (ii) from the surface of the posterior part of the pyriform cortex, the 'lateral cortico-habenular tract', which has been widely recognized in other reptiles and in amphibia (Herrick, 1948). The third contribution to the stria medullaris is the anterior olfacto-habenular tract, a compact bundle which can be followed forwards and dorsally near the surface of the paraterminal body (Text-figs. 3, 1 and 2 and Pl. 1, fig. 4) until it is lost over the anterior pole of the hemisphere. Here it comes into relationship with the hippocampal and dorsal cortical areas and the anterior olfactory nucleus. It should be noted that the terms 'cortico-habenular' and 'olfacto-habenular' both indicate that the fibres concerned run caudally to end in the habenular ganglia. This, of course, is uncertain, since the methods which have been used to demonstrate these tracts, at least in reptiles, can show only their topographical position and course. In one reptile, *Lacerta viridis*, (Gamble, 1952a), it has been shown by experimental degeneration that the 'olfacto-habenular tract' in fact runs in the opposite direction, that is from the region of the habenular ganglia cranially, and that it consists of secondary olfactory fibres which have crossed in the habenular commissure and probably have no functional connexion with the habenular ganglia. It was partly with the purpose of finding whether the olfacto-habenular tract in another and very different reptile was of a similar nature that the present investigation was undertaken.

Many other fibre tracts in the forebrain could of course be described, such as the stria terminalis, forebrain bundles, etc. The details are not relevant to the degeneration experiments to be reported, and the general position and characteristics of such tracts are sufficiently indicated in the Text-figs. 1-6.

Characteristics and distribution of experimental degeneration

It is well known that various morphological changes can be demonstrated by silver impregnation methods along the course and at the termination of degenerating axons. The character of these changes depends on a large number of factors; the species of the animal, the particular tract of fibres which is being studied, the length of time allowed for degeneration and the technical method employed, are among the most important of these. In the tortoise, and using the Nonidez technique, the most conspicuous changes were found in secondary olfactory fibres about 10 days after removal of the bulb (Pl. 1, figs. 2, 3). At this time affected fibres are fragmented, leaving a considerable quantity of argyrophilic granular debris along their course, which is easily recognizable and gives an appearance strikingly different from that of the normal tract (cf. Pl. 1, figs. 1, 2). It is probable that even at this stage some debris has been removed, and in specimens which have survived a few days longer this process has gone so much further that loss of fibres is the most conspicuous feature in the affected tracts (Pl. 1, fig. 4). It is obvious that this loss of fibres will be observable only if at least a considerable proportion are affected, and even then it will be necessary to control the observation by comparison with the normal condition. Clearly the most convenient and satisfactory control will be comparison with the normal tract on the opposite side of the same animal, but this will be possible only when the effects of the lesion are unilateral so far as the tract under consideration is concerned. After shorter survival times (5-8 days) degenerat-

ing olfactory tract fibres in the tortoise show numerous irregular varicosities, but little fragmentation. Since these varicosities are not found in the normal brain, and also since their situation corresponds exactly with that of the axonal debris and loss of axons which can be observed after longer periods of survival, there seems no doubt that they constitute reliable evidence of degeneration in the conditions of these experiments. 'Terminal degeneration', whether in pericellular arborizations or in end bulbs, has been frequently reported, and when seen can provide most valuable and precise information about fibre connexions. In the present experiments, however, nothing was seen which could be interpreted with confidence as terminal degeneration. This does not mean that they provide no information about the connexions of fibre tracts. If axonal debris or any other visible result of degeneration is traced until a point is reached where it can no longer be seen, it is a reasonable inference that the affected fibres end in synaptic junctions near that point, although the synapses themselves may not be observed directly. Such inferences, though generally reliable, lack the detailed precision which clear-cut terminal degenerative changes can provide.

The experimental lesions were all limited to the olfactory bulbs, except in two cases where some damage, apparently due to interference with blood vessels, was found in the ventral and anterior parts of the palaeostriatum and anterior olfactory nucleus. Since the degeneration in these two specimens did not differ in its distribution from that in the others, this additional damage was clearly not extensive enough to cause demonstrable degeneration. In general, the results in all animals were similar in the distribution of degeneration, although the amount naturally varied according to the extent of the lesion. Owing to the proximity of the accessory to the main bulb in most specimens both received damage. Damage appeared to be limited to the main bulb in one case only, where almost inevitably it was very incomplete. The experiments, therefore, allowed no distinction to be made between the connexions of the main and accessory olfactory bulbs.

According to the survival time, axonal debris or axonal varicosities and small bulbous swellings could be found throughout the extent of the lateral olfactory tract described above. It was widespread and abundant in the dorsal part of the tract over about the anterior half of the pyriform cortical area. Posteriorly, behind the level of the lamina terminalis where the tract is reduced in size and limited to the region of the nucleus of the lateral olfactory tract along the ventral border of the pyriform cortex, degeneration was similarly limited in its distribution. It could be traced further posteriorly to become widespread again over the central amygdaloid mass, and, to a smaller extent, to penetrate into its substance. In two cases (in both of which the lesion had included the accessory olfactory bulb) a few axonal varicosities and bulbous swellings were found in the posterior part of the hypopallium, but such changes could not be traced into continuity with the lateral olfactory tract, and can be regarded as no more than suggestive evidence that secondary olfactory fibres reach this part of the brain. It should be noted that no evidence of degeneration was found over about the posterior half of the pyriform cortex in any specimen.

The degeneration mentioned so far was limited to the operated side in specimens where the lesion was unilateral so that comparison of the two sides could be used as a control. The more interesting findings, however, were made in secondary olfactory

fibres which crossed to the opposite side. The ventral part of the lateral olfactory tract, which spreads over the olfactory tubercle, showed extensive degenerative changes, strongly suggestive of connexions with the cells of the tubercle. The great majority of these degenerating axons, however, pass posteriorly to enter the stria medullaris where they could be followed through the habenular commissure into the opposite stria. From here they separated into two major pathways: (i) through the length of the 'anterior olfacto-habenular tract' to its termination on the surface of the anterior extremities of the hippocampal and dorsal cortices and the anterior olfactory nucleus, and (ii) laterally across the ventral surface of the hemisphere behind the olfactory tubercle to the surface of the pyriform cortex in about the middle of its extent and to the more anterior part of the central amygdaloid mass. This second pathway corresponds closely to the 'lateral cortico-habenular tract' described by many authors. Very extensive axonal destruction in the stria medullaris from a specimen in which both olfactory bulbs were removed is shown in Pl. 1, figs. 2 and 3, and compared with the appearance of the stria in a normal brain (Pl. 1, fig. 1). Pl. 1, fig. 4, illustrates loss of fibres from the contra-lateral anterior olfacto-habenular tract of a specimen which survived unilateral bulbar ablation for 11 days.

From these findings one may conclude that the olfactory tract fibres described in the first part of this paper consist predominantly of axons which have had their origin in the main and accessory olfactory bulbs, presumably from the mitral cells, and that these fibres run to the anterior half of the pyriform cortex, the nucleus of the lateral olfactory tract and the amygdala, all on the same side. That they end in these structures is almost certain, although unequivocal 'terminal' degeneration has not been seen. The olfactory tubercle can be added to these structures as receiving homolateral secondary olfactory fibres, and possibly the posterior part of the hypopallium. The evidence for the last connexion is not entirely convincing, and in any case involves only a few fibres. The homo-lateral connexions described have all been inferred from an examination of normal material, and the experimental evidence is merely a confirmation of the inferences. Negatively, the absence of experimental confirmation for the presence of direct bulbar connexions in the posterior pyriform cortex is of some importance, and a similar absence of homo-lateral bulbar connexions for the hippocampal cortex and paraterminal body may be pointed out. It must be admitted, however, that the presence of a few scattered degenerated fibres might easily be missed, so that the apparent absence of bulbar connexions from these regions may be relative only.

The presence, however, of widespread hetero-lateral bulbar connexions could not be inferred from an examination of normal material with the techniques usually employed. The degeneration experiments make it clear that the stria medullaris, anterior olfacto-habenular tract and lateral cortico-habenular tract consist largely of secondary olfactory fibres which have crossed or are about to cross, and that the habenular commissure is an olfactory commissure. Since some fibres persist in all these situations after bilateral ablation all the fibres concerned are not of this nature, though probably the majority are.

DISCUSSION

These results may be compared with those in other reptiles from the point of view of the characteristics of the degeneration itself, and also from the point of view of the anatomical conclusions which can be reached.

So far as the degeneration itself is concerned, in *Lacerta viridis* (Gamble, 1952a) it was found that maximal formation of axonal varicosities was demonstrable in the olfactory pathways after 2 to 3 days' survival, and that granular axonal debris was never conspicuous and practically completely removed a few days later. This rapid and abrupt course of degeneration contrasted strongly with Armstrong's (1950) findings in the visual pathways of *L. vivipara* when survival periods of 2 weeks provided maximal evidence of axonal degeneration, and where debris persisted for as long as 2 months. Similar experiments in *Natrix natrix* (Armstrong, 1951) gave evidence of the persistence of axonal debris for as long as 4 months in some specimens. Obviously no general conclusions can be based on these observations; they emphasize, however, the variability, particularly in the time relationships, of degeneration in different species and different fibre systems.

The experimental degenerations which confirm inferences made from the study of normal material call for little comment, and this applies to almost all the homolateral findings. The absence (or relative absence) of bulbar connexions in the posterior pyriform cortex may be significant in relation to the observation that such connexions are also absent in the posterior pyriform or entorhinal area of mammals (Clark & Meyer, 1947; Meyer & Allison, 1949). The crossed connexions, however, are in a different category. They have previously been described only in *Lacerta viridis* (Gamble, 1952a, b); and although in other reptiles the stria medullaris, anterior olfacto-habenular and lateral cortico-habenular tracts exist and pursue similar courses, that they should be composed of secondary olfactory fibres seemed, on the face of it, so unlikely that experimental investigation of their nature in at least one other reptile was desirable. Direct evidence that they do consist of crossing secondary olfactory fibres as in *Lacerta*, seems now to be adequate, and greatly strengthens the case for believing that such pathways are common to all reptiles.

The possible relationship between the habenular-crossing fibres of *Lacerta* and similar though differently named tracts in Amphibia has been discussed already (Gamble, 1952a, b) and needs no further elaboration. Possible relationships with similar tracts in mammals were also discussed, but two points may now be considered further.

It was noted that the 'stria terminalis 5' component of the stria medullaris of the opossum (Loo, 1931) very closely resembled the anterior olfacto-habenular tract of *Lacerta*; equally it resembles that of *Testudo*. Other less striking comparisons may also be made. The 'anterior olfacto-habenular tract' and the 'lateral cortico-habenular tract' of the opossum differ from the similarly named tracts of *Testudo* only slightly. In the opossum both join the stria by passing medial to the internal capsule, while in *Testudo* they join it by passing lateral to the lateral forebrain bundle. It is also true that Loo's 'anterior olfacto-habenular tract', as such, extends only to the olfactory tubercle, but it is so intermingled with his 'tractus olfacto-hypothalamicus ventralis' that its precise extent could not be elucidated in normal material. It is certainly possible that the condition found in *Lacerta* and *Testudo*

may exist in the opossum, and the similarity in the arrangement of the components of the stria suggests a probability that this is so.

The secondary olfactory connexions in the phalanger have been investigated experimentally by Adey (1953). In general his findings accorded closely with those reported in the rabbit (Clark & Meyer, 1947) and in the monkey (Meyer & Allison, 1949), but with one exception possibly relevant in the present context. He described axonal degeneration in one component of the stria medullaris. This component, the 'periventricular olfacto-habenular tract' is the one which is least like the crossing olfactory components of the stria of *Testudo*, but the presence of any component of bulbar origin is of interest. It would be interesting to know whether or not similar degeneration occurred also on the opposite side and in the habenular commissure.

The finding of widespread and extensive terminal degeneration in the bed nucleus of the stria terminalis and certain of the amygdaloid nuclei of the unoperated hemisphere has been common to all recent experimental investigations of secondary olfactory connexions in mammals so that the problem of where crossing occurs is raised. The axons whose terminals have been seen to degenerate have not themselves been identified throughout their course. Axonal degeneration has, it is true, sometimes been reported in the anterior commissure; but in the phalanger 'the anterior commissure forms an exceedingly large bundle, but degenerating fibres are very few' (Adey): and in the rabbit, when the lesion definitely excluded the anterior olfactory nucleus 'relatively few fibres of the anterior commissure were found to be degenerated' (Clark & Meyer). In the monkey, although the anterior commissure is said to be the pathway used by the crossing fibres, no evidence of this has been presented (Meyer & Allison, 1949). Indeed the only evidence directly bearing on this problem is the result of one experiment in the rabbit described by Allison (1953) where terminal degeneration was reported bilaterally in the bed nucleus of the stria terminalis following transection of the anterior limb of the anterior commissure. Even here possible damage to the olfactory tract cannot be excluded with certainty, and some degeneration was also present in the outer layers of the prepyriform cortex. While a crossing in the anterior commissure may be the likeliest pathway for these secondary olfactory fibres, it can hardly be considered proven, and the present findings suggest that a course through the habenular commissure should be considered.

In reptiles which have been experimentally investigated, the 'olfactory component of the anterior commissure' either does not exist, e.g. *Testudo*, or is wholly unaffected by removal of the olfactory bulbs, e.g. *Lacerta* (Gamble, 1952*b*). Equally, in both species there is no evidence that interbulbar fibres exist. Both these features appear to be mammalian characteristics, and to be related to the presence of characteristically 'tufted' cells in the mammalian olfactory bulb (Allison, 1953). Crossing olfactory fibres which undoubtedly exist in reptiles must, in the absence of 'tufted' cells, be the axons of mitral cells and they cross in the habenular commissure. It is reasonable to suppose that similar pathways may exist in mammals in addition to new interbulbar fibres in the anterior commissure; the constancy of the pattern formed by the components of the stria medullaris in reptiles and mammals could alone provide a *prima facie* case for such a hypothesis.

SUMMARY

Degenerative changes in secondary olfactory fibres following experimental lesions in the olfactory bulbar apparatus of *Testudo graeca* have been traced in serial silver impregnated sections. An essentially similar pattern to that described in *Lacerta viridis* (Gamble, 1952*a*) has been demonstrated. Its possible relevance to the pattern of secondary olfactory fibres in mammals has been discussed, with particular emphasis on those components which transverse the stria medullaris and cross in the habenular commissure.

I am greatly indebted to Prof. F. Goldby for his advice and criticism during the course of this work, and to Mr R. Jarrett who prepared one of the photographs.

REFERENCES

- ADEY, W. R. (1953). An experimental study of the central olfactory connexions in a Marsupial (*Trichosurus vulpecula*). *Brain*, **76**, 311-330.
- ALLISON, A. C. (1953). The structure of the olfactory bulb and its relationship to the olfactory pathways in the rabbit and the rat. *J. comp. Neurol.* **98**, 309-353.
- ARMSTRONG, J. A. (1950). An experimental study of the visual pathways in a reptile (*Lacerta vivipara*). *J. Anat., Lond.*, **84**, 146-167.
- ARMSTRONG, J. A. (1951). An experimental study of the visual pathways in a snake (*Natrix natrix*). *J. Anat., Lond.*, **85**, 275-288.
- CLARK, W. E. LE GROS & MEYER, M. (1947). The terminal connexions of the olfactory tract in the rabbit. *Brain*, **70**, 304-328.
- CROSBY, E. C. & HUMPHREY, T. (1939). Studies on the vertebrate telencephalon. I. The nuclear configuration of the olfactory and accessory olfactory formations and the nucleus olfactorius anterior of certain reptiles, birds and mammals. *J. comp. Neurol.* **71**, 121-213.
- GAMBLE, H. J. (1952*a*). An experimental study of the secondary olfactory connexions in *Lacerta viridis*. *J. Anat., Lond.*, **86**, 180-196.
- GAMBLE, H. J. (1952*b*). An experimental study of the secondary olfactory connexions of *Lacerta viridis*. M.Sc. Thesis, University of London.
- GOLDBY, F. (1936). A study of the comparative anatomy of the reptilian hemisphere in relation to the evolution of structure and function in the mammalian brain. M.D. Thesis, University of Cambridge.
- GOLDBY, F. (1937). An experimental investigation of the cerebral hemispheres of *Lacerta viridis*. *J. Anat., Lond.*, **71**, 332-355.
- HERRICK, C. J. (1948). *The Brain of the Tiger Salamander*. University of Chicago Press.
- JOHNSTON, J. B. (1915). Cell masses in the forebrain of the turtle, *Cistudo carolina*. *J. comp. Neurol.* **25** 393-468.
- JOHNSTON, J. B. (1923). Further contributions to the study of the evolution of the forebrain. *J. comp. Neurol.* **35**, 337-481.
- LOO, Y. T. (1931). The forebrain of the opossum, *Didelphis virginiana*. *J. comp. Neurol.* **52**, 1-148.
- MEYER, M. & ALLISON, A. C. (1949). An experimental investigation of the connexions of the olfactory tracts in the monkey. *J. Neurol. Psychiat.* **12**, 274-286.
- NONIDEZ, J. F. (1939). Quoted by Lillie, R. D. (1948). In *Histopathologic Technic*. Philadelphia: Blakiston Co.
- SCHEPERS, G. W. H. (1948). *Evolution of the Forebrain*. Cape Town: Maskew Miller Ltd.

TERMINAL DEGENERATION IN THE DIENCEPHALON AFTER ABLATION OF FRONTAL CORTEX IN THE CAT

By J. AUER

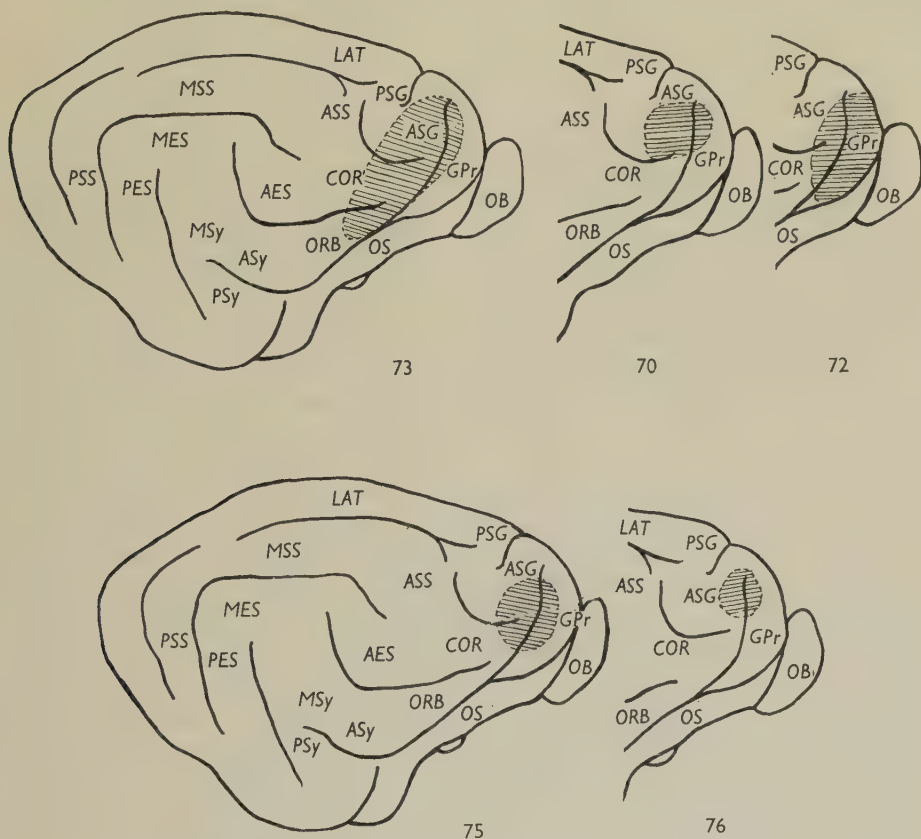
Department of Anatomy, University of Ottawa, Canada

Corticofugal connexions arising from the frontal lobe have been reported coursing to various nuclei in the diencephalon. Particular reference should be made in this respect to the work of Le Gros Clark (1932), Le Gros Clark & Boggon (1935), Mettler (1935), Meyer (1949) and Wall, Glees & Fulton (1951). The earlier findings stem from Marchi material. Meyer studied human material with a modified Bielschowsky technique (Glees, 1946), and so did Wall and his co-workers in the rhesus monkey. However, only few observations are in accordance with the recent data derived from neurophysiological experiments (Niemer & Jimenez-Castellanos, 1950; Stratford, 1954; Jasper, Ajmone-Marson & Stoll, 1952; Livingston, Hernández-Péon & French, 1953). It should be emphasized that particularly the so-called unspecific thalamic nuclei have been little investigated with a view to revealing terminals from cortical efferents. Neither the Marchi technique nor the modified Bielschowsky method (Glees, 1946) seem to be suitable for this purpose. The former does not show unmyelinated fibres which are abundant in those nuclei while the latter does allow precise detection, only after considerable exploration of the various fields.

The present study was primarily carried out in order to examine cortical efferents from the frontal lobe to the hypothalamus. It had been impossible in recent studies (Auer & di Virgilio, 1953) to verify several connexions inferred (Ward & McCulloch, 1947) and described earlier (Le Gros Clark, 1948; Meyer, 1949). In view of the fact that a bouton technique (Auer & di Virgilio, 1953) was used primarily for this purpose, it was thought advisable to study the same problem with a selective silver impregnation technique (Nauta & Gyax, 1954) for degenerating fibres and terminals. However, observations on all specific and non-specific diencephalic nuclei, particularly those nuclei that have recently been discussed as the diffuse thalamic projection system (Jasper, 1949; Hanbury & Jasper, 1953; Starzl & Magoun, 1951), were included in the investigation. It was considered important to study the counterpart of the problem of the connexions from the thalamus to the cortex which have recently been clarified by the investigations of Rose (1952) and Powell & Cowan (1954). Therefore, the experiments described below deal with observations on efferents to (a) the specific thalamic nuclei, (b) the unspecific thalamic nuclei, (c) the subthalamus and (d) the hypothalamic nuclei. The subthalamus is also included in view of its intimate relationship with the nucleus reticularis which, as recent evidence (Droogleever Fortuyn, 1950) has shown again, belongs to the unspecific thalamic nuclei.

MATERIAL AND METHODS

A series of five cats from a larger group has been used for this investigation. The operations were performed under antiseptic conditions and nembital anaesthesia. A round opening was made with a drill in the roof of the right frontal sinus. The postero-inferior wall of this sinus was then removed in order to expose the frontal part of the brain. The right cruciate sulcus could usually be identified easily and a



Text-fig. 1. Site of cortical lesion in cats 73, 70, 72, 75 and 76.

lesion was inflicted with a long thin thermocauter blade to the area anterior to this sulcus. As far as possible the attempt was made to restrict the lesion to the gyrus prefrontalis and its immediate surrounding. The amount of bleeding encountered was usually negligible and the exposed area was closed by a filling of bone wax. A double layer of sutures was used to close the surface wound. Infections were never seen and the animals were all sacrificed by the fifth or sixth day after the operation. Functional changes during the post-operative period were never observed, due mainly to the fact that the lesions were unilateral. Text-fig. 1 illustrates the different sites of these lesions in cats, 73, 70, 72, 75 and 76.

The five specimens were fixed in neutral formalin after perfusion with physiological saline. Serial sections were subsequently stained with the Nauta-Gygax technique (1954) for selective impregnation of degenerating fibres of passage and terminals. This method has proved to be much more reliable than that used earlier for detection of terminal boutons (Auer & di Virgilio, 1953). The latter method is only of value for the study of a restricted area where changes are anticipated beforehand. Some brains stained with this method were used for control of the findings in the present series. Orientation of the sections was greatly facilitated by the use of an atlas on the diencephalon of the cat (Jasper & Ajmone-Marsan, 1954).

OBSERVATIONS

The brain of cat 73 shows the most extensive lesion in the cortex, penetrating slightly into the white matter. Practically the whole anterior sigmoid gyrus has been damaged and also the most anterior tip of the coronary gyrus and the orbital gyrus. As Text-fig. 1 illustrates a narrow strip of the gyrus proreus was also included in the lesion. The degenerating fibres can be followed in the internal capsule and can be seen entering into the diencephalon via the anterior thalamic peduncle as well as through the inferior thalamic peduncle. The fibres of the former are piercing the head of the reticular nucleus. This nucleus shows a large number of degenerating terminals (see Pl. 1). However, some fibres are seen traversing this nucleus and they gradually disappear in more caudal areas of the thalamus, mainly in the intralaminar nuclei. This cat which, as has been said above, contains the most extensive cortical lesion of the series, does not show any terminal degeneration in the nucleus ventralis anterior. The anterior group of nuclei is also entirely devoid of signs of fibre and terminal degeneration. The traversing fibres reach the intralaminar nuclei of which the nucleus centralis lateralis and the nucleus paracentralis should be emphasized as the end-stations. Other fibres reach the nucleus ventralis lateralis in which there is extensive terminal degeneration. More posteriorly the nucleus ventralis postero-medialis (Text-fig. 2; Pl. 2, fig. 2) the nucleus ventralis medialis and on the same level the caudal extent of the nucleus ventralis lateralis show extensive terminal degeneration. Most of these terminals arise from fibres entering through the lateral part of the reticular nucleus. The medial nuclei at this level have been explored in detail. The nucleus dorsalis medialis as well as the nucleus centralis medialis (Text-fig. 2) have suffered less from terminal degeneration than, for instance, the nucleus ventralis posteromedialis. The nucleus dorsalis medialis possesses only a few degenerating terminals, apparently entering into the nucleus from the internal medullary lamina. It is interesting to note that terminal degeneration in the nucleus centralis medialis was also observed in similar experiments after the use of a bouton technique (Auer & di Virgilio, 1953). Analysis of observations in the subthalamic area and the centrum medianum is somewhat more difficult. Degenerating fibres of passage are present on each side of the zona incerta (Text-fig. 2; Pl. 2, fig. 7). The latter area possesses degenerating terminals. This is also seen in the subthalamic nucleus and the entopeduncular nucleus. It is a matter of some speculation at present as to how the centrum medianum receives its degenerating fibres. It would appear that most of them enter via the fasciculi thalamicus and lenticularis. These

bundles also send many fibres towards the reticular formation immediately caudal to the centrum medianum (Text-fig. 2).

Since only the caudal strip of the gyrus proreus has been damaged the hypothalamus does not show conspicuous changes. However, the medial forebrain

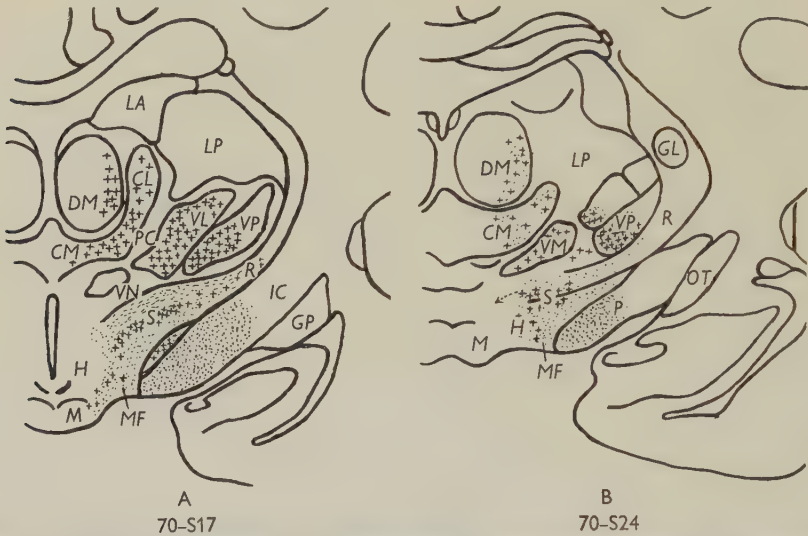


Text-fig. 2. Diagram of localization of degeneration in caudal diencephalon of cat 73. Crosses represent degenerating terminals; dots represent degenerating fibres of passage. Dotted arrow, to tegmentum; lined arrow, to hypothalamus.

bundle contains scattered fibres of passage showing degeneration, and degenerating terminals are seen originating from these fibres. An undeniable verification of connexions to the ventromedial nucleus and the mamillary nuclei as inferred from physiological experiments in the monkey (Ward & McCulloch, 1947) was not obtained in this cat (Text-fig. 2).

Cat 70

The brain of cat 70 shows a cortical lesion which is slightly smaller than in cat 73. The degeneration in the diencephalon, however, is very similar. Degenerating terminals among the incoming fibres of passage are especially noted in the head of the reticular nucleus. The density of the degeneration, however, is slightly less than in cat 73. The lateral part of the nucleus dorsalis medialis as well as the adjacent nuclei (Pl. 1, fig. 3) of the internal medullary lamina present degenerating terminals as also seen in cat 73. The degenerating fibres directed towards the nucleus ventralis lateralis



Text-fig. 3. Diagram of localization of degeneration in diencephalon of cat 70. Crosses represent degenerating terminals; dashes and dots represent degenerating fibres of passage.

and ventralis posterior traverse through the lateral part of the nucleus reticularis in which scattered terminal degeneration is noticeable. It should be pointed out that the head of the reticular nucleus, the lateral part of this nucleus and the continuation of this structure into the subthalamus contain degeneration throughout, degenerating fibres of passage as well as terminals. However, the greatest concentration of terminal degeneration in all cats examined is present in the head of the reticular nucleus. The hypothalamus in this specimen is characterized by degenerating fibres traversing the medial forebrain bundle; this is particularly noticeable in the caudal part of the hypothalamus between the mamillary body and the internal capsule (Text-fig. 3). The anterior part of the mamillary body contains several degenerating terminals. The origin of these terminals seems to lie in the medial forebrain bundle. Scattered terminal degeneration is present in the nucleus ventro-medialis. The question must be raised whether a few degenerating terminals are significant enough to enable a conclusion to be made. However, comparison with the normal side shows a difference which renders it justifiable to mention the presence of degenerating terminals in some hypothalamic nuclei. The

subthalamus shows exactly the same sort of picture as described for cat 73. This region, particularly the fields of Forel, are thoroughfares which are crowded with degenerating fibres in all five of the cats.

Cat 72

The brain of cat 72 has a lesion which completely destroyed the right gyrus proreus. Only a small rostral strip of the anterior sigmoid gyrus was damaged by the instrument. Some white matter deep to the cortex of the gyrus proreus was also damaged. The head of the reticular nucleus is again filled with numerous degenerating terminals and fibres of passage in degeneration. This part of the nucleus also contains a few degenerating terminals at the left side, which can only be due to the fact that a very small lesion was present at the medial side of the left gyrus proreus, inflicted by the tip of the blade. The degenerating terminals in the nuclei centralis lateralis, paracentralis and centralis medialis, offer a picture similar to the one described above (Text-fig. 4).

It would appear that a topic difference in efferents, i.e. a difference in distribution, from the gyrus proreus and the sensorimotor cortex, cannot be detected with the method used; it remains to be seen whether such a difference exists. Only the concentration of the degeneration seems to vary with the size of the lesion. A typical predilection is noted for the head of the reticular nucleus, which is the preferred site in that nucleus for diffuse efferents from the frontal lobe.

The inferior thalamic peduncle contains many degenerating fibres of passage which seem to be directed towards the dorsomedial nucleus and the intralaminar nuclei (Pl. 1, fig. 4). It seems impossible to accept the conclusion of Bodian (1940) that this peduncle carries mostly striate and pallidal connexions to the thalamus.

The specific nuclei of the thalamus in this cat offer the same picture as described for cats 70 and 73, with respect to the nucleus ventrolateralis and ventralis postero-medialis. However, the concentration of the degeneration is much less, due to the lesser involvement of the sensori-motor cortex in the lesion. This is not the case with the dorsomedial nucleus, which shows the heaviest concentration of degenerating terminals in the whole series (Text-fig. 4; Pl. 2, fig. 5). The lateral half of the nucleus is particularly affected. The forward displacement of the lesion in this experiment has definitely brought about a medial displacement of terminal degeneration in the specific nuclei.

The hypothalamus has been thoroughly investigated in this cat because of the complete destruction of the gyrus proreus at the right side. The lateral hypothalamic area possesses numerous degenerating terminals along the medial forebrain bundle. As in the former case a slight difference exists indeed between the right and left infundibular and mamillary regions. A few degenerating terminals are seen at the right side in the ventromedial, the lateral mamillary and the posterior hypothalamic nucleus. These terminals apparently arise from the medial forebrain bundle (Text-fig. 4).

Cats 75 and 76

The brains of these specimens may be described together as their lesions are approximately of the same size; they are added to the description in order to provide additional evidence to the data described for the other experiments. The brain of

cat 76 has the smaller lesion of the two, and it is doubtless because of this that a lesser number of degenerating terminals is noticed in the head of the reticular nucleus. The concentration of degenerating terminals in the nucleus ventralis lateralis and nucleus ventralis posterior is less than in any of the other experiments. This also



[Text-fig. 4. Diagram of degeneration in diencephalon of cat 72. Crosses represent terminal degeneration, dashes and dots represent degenerating fibres of passage.

applies to the intralaminar nuclei and the nucleus centralis medialis. The number of abnormal terminals in the dorsomedial nucleus is so few that the degeneration cannot be considered significant after comparison with the brains described above. The brain of cat 75 showed some change to the white matter which resulted in destruction of more fibres arising from the gyrus preureus than would be expected from the

size of the lesion. This is doubtless why the dorsomedial nucleus shows more extensive degeneration than in cat 76.

Both brains have extensive degeneration in the subthalamus. Fibres of passage in degeneration are noticed and can be followed to the centrum medianum and to the midbrain tegmentum (Pl. 2, fig. 8). The grey matter of these areas obviously receives fibres from the damaged cortical area as has been described above for the first three brains.

The hypothalamus shows some fibre degeneration only in cat 75; however, these observations are less significant even than those described above. Therefore conclusions will not be drawn from these last two experiments.

DISCUSSION AND CONCLUSION

The foregoing observations provide ample evidence for the acceptance of numerous connexions in the cat's brain from the sensori-motor cortex and the prefrontal cortex (gyrus proreus) to the reticular nucleus, the intralaminar nuclei, the centrum medianum and the midline nuclei of the thalamus.

An anatomical substratum is hereby provided for the physiological data reported by Livingston *et al.* (1953). Reference may be made also to afferent connexions, recently established, to the rostral telencephalon from some unspecific nuclei (Rose, 1952; Powell & Cowan, 1954) in order to assume the existence of 'unspecific' thalamo-cortical circuits coursing through the diffusely projecting thalamic nuclei. How such circuits are to be related to the earlier established specific circuits (Hsiang-tung Chang, 1950) remains to be investigated. Anatomical data is now accumulating to show how these unspecific thalamic nuclei can be brought to bear upon the whole cortex by stimulation, not only from afferent systems (Starzl, Taylor & Magoun, 1951), but also from the cortex; that is to say, the cortex of the frontal lobe, as far as the present study is concerned. It may be asked, for instance, how the visual cortex projects on these unspecific nuclei, in view of the hypothesis proposed by Livingston *et al.* (1953) on the role played by the system as a whole with regard to sustained attention. Such connexions from the visual cortex were not reported by Nauta & Bucher (1954) in the rat. These authors, however, reported abundant connexions from the visual cortex to the reticular formation of the brain stem. Similar connexions are also arising from the damaged cortex in the experiments reported above, but their detailed description has not been added to this paper. It seems difficult to maintain the rather artificial separation of the reticular formation of the midbrain from the intralaminar and midline nuclei of the thalamus, in the light of the accumulating evidence that it forms a functional unit with these nuclei of the diencephalon. The connexions to the dorsomedial nucleus, already reported by Le Gros Clark (1932, with the Marchi technique) and more recently by Meyer (1949, in leucotomy material with a modified Bielschowsky technique) have now also been revealed with the selective silver impregnation method used in this study. These connexions seem to enter with the anterior and inferior thalamic radiation together with most of the fibres directed to the nuclei centralis lateralis, paracentralis and centralis medialis. It seems important that, in the cat, lesions in the cortex of the gyrus proreus result in degeneration mainly in the lateral and ventral

parts of this nuclear group. The present study failed to reveal changes in the medial part. It should be mentioned here that the most recent evidence on efferent connexions from the gyrus proreus (Stratford, 1954) did not show abundant terminals in these nuclei. However, these negative results may be due to the strychnine neuronography used by this author. The present findings are in accordance with the physiological observations, also on cat material, carried out by Niemer & Jimenez-Castellanos (1950).

The attention drawn to the nucleus ventralis anterior by some recent physiological studies (e.g. Starzl & Magoun, 1951) could not be justified by the present investigation. It is no doubt true that some fibres of passage traverse this nucleus, but terminal degeneration has not been observed in it.

It would be interesting to compare the present studies with material obtained after destruction of the head of the caudate nucleus. Degeneration in the intralaminar nuclei and changes in the nucleus ventralis anterior would then seem to be in line with earlier investigations with physiological methods (Shimamoto & Verzeano, 1954). At present one can only conclude that the nucleus ventralis anterior is not a reception area for diffuse terminals from the frontal cortex, but it may be a relay nucleus between the thalamus and the caudate nucleus. The findings in the nucleus ventralis lateralis and ventralis posterior are only a confirmation of earlier studies on these nuclei, particularly by Gerebtzoff & Wauters (1941). It is, however, in the light of the observations of Niemer & Jimenez-Castellanos (1950), interesting to note that the nucleus ventralis posterior also receives connexions from the precruciate, i.e. the motor cortex.

The fact that the subthalamus was never clear of degenerating fibres emphasizes the conclusion that this area may be classified with the intralaminar and reticular nuclei as belonging to one system which receives diffusely arranged corticodiencephalic fibres. It is quite correct to state with Niemer & Jimenez-Castellanos (1950) that the subthalamus represents a 'strategic bottleneck'. It should be emphasized strongly that the head of the reticular nucleus as well as its caudal continuation (Droogleever Fortuyn, 1950) show these degenerating terminals more abundantly than any other part of the same extralaminar structures. It is not yet possible to analyse further from which regions of the cortex the different elements of the subthalamus receive terminals, and similarly an analysis in this respect of the fasciculi thalamicus and lenticularis cannot be offered. The lesions under consideration are in the first place too extensive for this purpose. However, the distribution of fibres in the whole area of the subthalamus after different ablations justify the conclusion that the efferents observed here have a rather diffuse spread without any topic arrangement whatsoever. The efferents to the hypothalamus have caused considerable difficulties, particularly in view of the evidence of earlier anatomical and physiological studies (Meyer, 1949; Ward & McCulloch, 1947). The present experiments caused degeneration in the medial forebrain bundle. This bundle showed degenerating fibres of passage as well as degenerating terminals, particularly in cats 70, 72 and 73. The number of degenerating terminals in the nucleus ventromedialis, the nucleus posterior and the nucleus mamillaris lateralis, observed in cats 70 and 72, is so small that one hardly feels justified in stating specifically that efferents to these nuclei are present. If one compares the data of Niemer & Jimenez-Castellanos (1950)

on the cat with the conclusions of Ward & McCulloch (1947) on the monkey one would be inclined to assume that there is a difference in the number of connexions in favour of the monkey. Meyer's illustrations (1949) show even more connexions in the leucotomy material of humans. This would lead to the conclusion that the number of cortical efferents to the hypothalamus has increased in higher mammals. Quantitatively such an increase is perhaps to be expected, since the area from which cortical efferents to the hypothalamus may arise in monkey and man is considerably larger than the gyrus preceus of the cat. This conclusion, however, needs further investigation, particularly qualitatively.

SUMMARY

Ablations were made in the precruciate and prefrontal cortex of five cats. The cats were sacrificed five to six days post-operatively, the brains were fixed in neutral formalin, subsequently sectioned, and stained with a selective impregnation technique for degenerating fibres of passage and terminals. Cortical efferents to the diencephalon have been observed and classified as follows:

(1) Diffuse efferents from the damaged areas are directed towards the intralaminar nuclei, reticular nucleus and centrum medianum.

(2) The dorsomedial nucleus shows terminal degeneration to an amount proportional to the size of the lesion in the gyrus preceus.

(3) The subthalamus has terminal degeneration in the zona incerta, and the subthalamic nucleus. Moreover, this area appears to be a thoroughfare for fibres of passage to the midbrain reticular formation and probably also to the centrum medianum.

(4) The hypothalamus is characterized by terminal degeneration and degenerating fibres of passage in the medial forebrain bundle. Little degeneration was observed in the ventromedial, the mammillary and the posterior hypothalamic nuclei.

Finally, confirmation has been obtained of cortical efferents to specific thalamic nuclei from the frontal lobe.

This work was supported by a grant in aid of research from the National Research Council of Canada.

REFERENCES

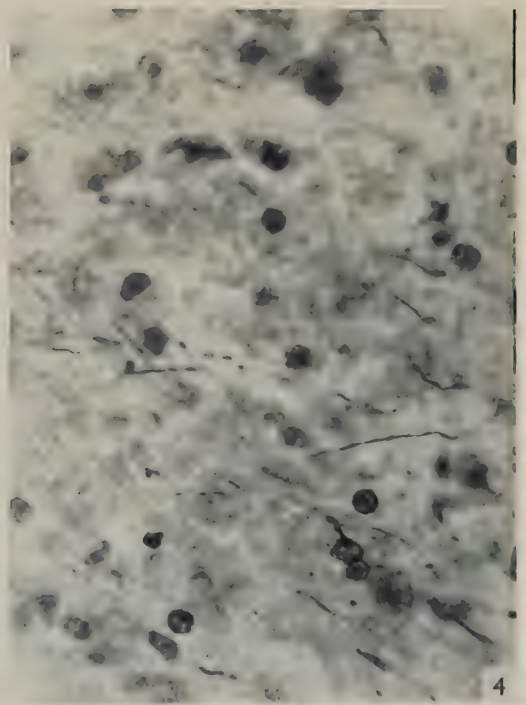
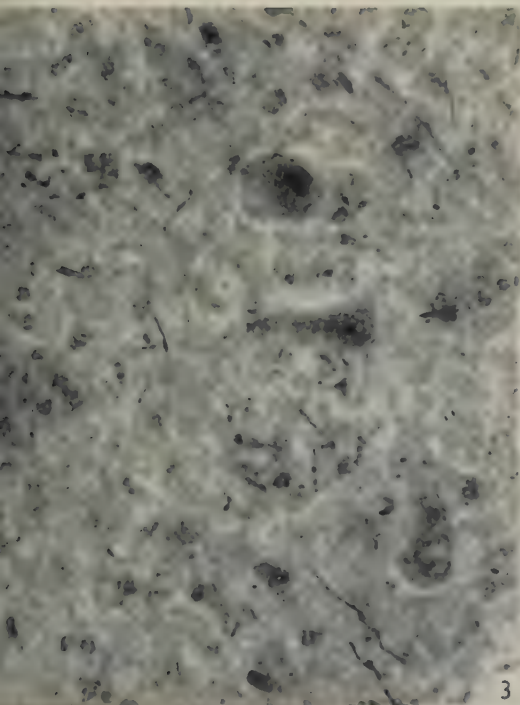
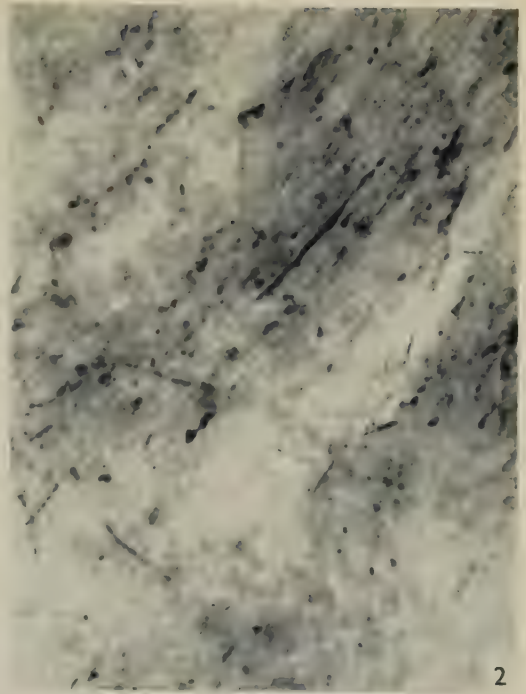
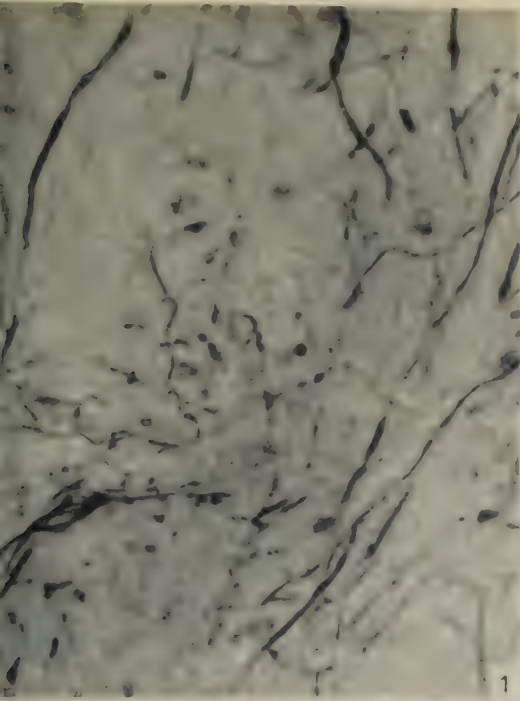
- AUER, J. & DI VIRGILIO, G. (1953). Some afferent connections of the hypothalamus in the cat. *Anat. Rec.* **115**, 277.
- AUER, J. & DI VIRGILIO, G. (1953). Demonstration of degenerating nerve fibers by a modified Cajal technique. *Stain Tech.* **28**, 141-146.
- BODIAN, D. (1940). Studies on the diencephalon of the Virginia opossum. II. The fiber connections in normal and experimental material. *J. comp. Neurol.* **72**, 207-297.
- DROOGLEEVER FORTUYN, J. (1950). On the configuration and the connections of the medio-ventral area and the midline cells in the thalamus of the rabbit. *Folia psychiat. neerl.* **53**, 213-254.
- GEREBTZOFF, M. A. & WAUTERS, A. (1941). Recherches sur l'écorce cérébrale et le thalamus du cobaye. II. Systématisation cortico-thalamique et voies efférentes de l'écorce cérébrale. *Cellule*, **48**, 7-70.
- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath.* **5**, 54-59.
- HANBURY, J. & JASPER, H. H. (1953). Independence of diffuse thalamocortical projection system shown by specific nuclear destruction. *J. Neurophysiol.* **16**, 252-272.

- HSIANG-TUNG CHANG (1950). The repetitive discharge of cortico-thalamic reverberating circuit. *J. Neurophysiol.* **13**, 235-258.
- JASPER, H. H. (1949). Diffuse projection systems: the integrative action of the thalamic reticular system. *Electroenceph. clin. Neurophysiol.* **1**, 405-419.
- JASPER, H. H. & AJMONE-MARSAN, C. (1954). *A Stereotaxic Atlas of the Diencephalon of the Cat*. Ottawa, Canada: Nat. Res. Council Publ.
- JASPER, H. H., AJMONE-MARSAN, C. & STOLL, J. (1952). Cortico-fugal projections to the brain stem. *Arch. neurol. Psychiat., Chicago*, **67**, 155-171.
- LE GROS CLARK, W. E. (1932). Structure and connections of the thalamus. *Brain*, **55**, 406-470.
- LE GROS CLARK, W. E. (1948). The connections of the frontal lobes in the brain. *Lancet*, **1**, 353-56.
- LE GROS CLARK, W. E. & BOGGON, R. H. (1935). The thalamic connections of the parietal and frontal lobes of the brain in the monkey. *Phil. Trans. B*, **224**, 313-359.
- LIVINGSTON, R. B., HERNÁNDEZ-PÉON & FRENCH, J. D. (1953). Corticofugal projections to brain stem activating system. *Fed. Proc.* **12**, 89.
- METTLER, F. A. (1935). Corticofugal fiber connections of the cortex of *Macaca muatta*. The frontal region. *J. comp. Neurol.* **61**, 509-542.
- MEYER, M. (1949). A study of efferent connections of the frontal lobe in the human brain after leucotomy. *Brain*, **72**, 265-297.
- MORRISON, R. S. & DEMPSEY, E. W. (1942). A study of thalamocortical relations. *Amer. J. Physiol.* **135**, 281-292.
- NAUTA, W. J. H. & BUCHER, V. M. (1954). Efferent connections of the striate cortex in the albino rat. *J. comp. Neurol.* **100**, 257-287.
- NAUTA, W. J. H. & GYGAX, P. A. (1954). Silver impregnation of degenerating axons in the central nervous system. *Stain Tech.* **29**, 91-93.
- NIEMER, W. T. & JIMENEZ-CASTELLANOS, J. (1950). Cortico-thalamic connections in the cat as revealed by 'physiological neuronography'. *J. comp. Neurol.* **93**, 101-125.
- POWELL, T. P. S. & COWAN, W. M. (1954). The connections of the midline and intralaminar nuclei of the thalamus of the rat. *J. Anat., Lond.*, **88**, 307-319.
- ROSE, J. E. (1952). The cortical connections of the reticular complex of the thalamus. *Res. Publ. Ass. nerv. ment. Dis.* **30**, 455-479.
- SHIMAMOTO, T. & VERZEANO, M. (1954). Relations between caudate and diffusely projecting thalamic nuclei. *J. Neurophysiol.* **17**, 278-289.
- STARZL, T. E. & MAGOUN, H. W. (1951). Organisation of the diffuse thalamic projection system. *J. Neurophysiol.* **14**, 133-147.
- STARZL, T. E., TAYLOR, C. W. & MAGOUN, H. W. (1951). Collateral afferent excitation of reticular formation of brain stem. *J. Neurophysiol.* **14**, 479-497.
- STRATFORD, J. (1954). Cortico-thalamic connections from gyrus proreus and first and second sensory areas. *J. comp. Neurol.* **100**, 1-15.
- WALL, P. D., GLEES, P. & FULTON, J. F. (1951). Corticofugal connections of posterior orbital surface in Rhesus monkey. *Brain*, **74**, 66-71.
- WARD, A. A. & McCULLOCH, W. S. (1947). The projection of the frontal lobe on the hypothalamus. *J. Neurophysiol.* **10**, 309-315.

EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Degeneration of terminals in the head of the nucleus reticularis (centre of picture). Section 17, cat 73; $\times 1250$.
- Fig. 2. Terminal degeneration and degenerating fibres of passage in the head of the nucleus reticularis. Section 10, cat 72; $\times 1250$.
- Fig. 3. Terminal degeneration in the nucleus centralis lateralis (centre of picture). Section 17, cat 70; $\times 1250$.
- Fig. 4. Scattered terminal degeneration in the nucleus paracentralis. Section 19, cat 72; $\times 1250$.



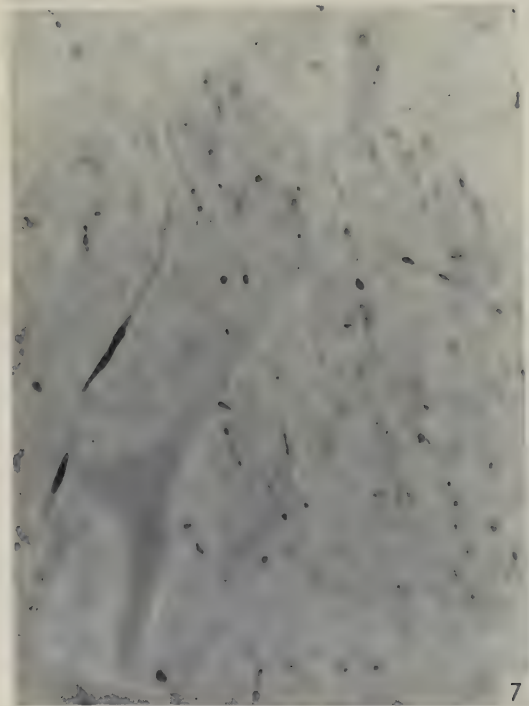
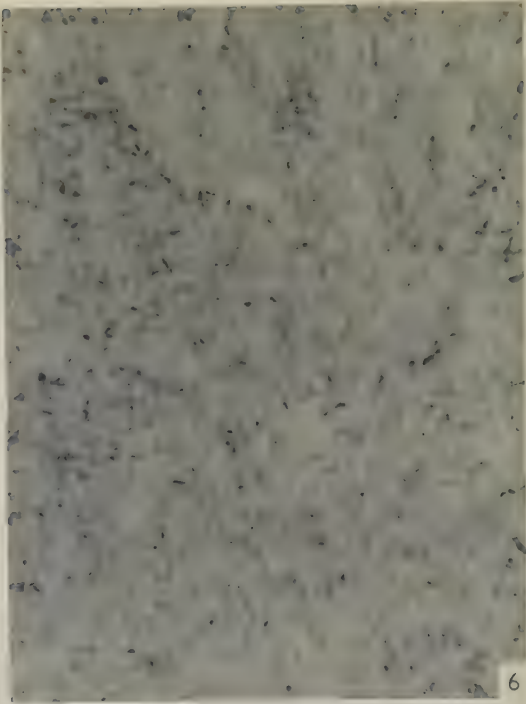
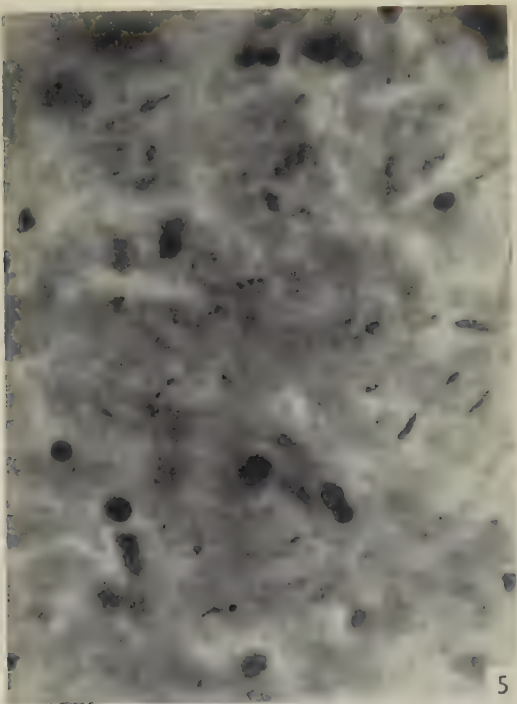


PLATE 2

Fig. 5. Degenerating terminals in the nucleus dorsomedialis thalami. Section 18, cat 72; $\times 1250$.

Fig. 6. Concentrated terminal degeneration in the nucleus ventralis posteromedialis. Section 28, cat 73; $\times 625$.

Fig. 7. Degenerating terminals and fibers in the zona incerta. Section 28, cat 73; $\times 1250$.

Fig. 8. Degenerating fibers of passage entering midbrain tegmentum from pes pedunculi. Section 11, cat 75; $\times 1250$.

KEY TO LETTERING

<i>AES</i>	anterior ectosylvian gyrus	<i>MES</i>	middle ectosylvian gyrus
<i>ASY</i>	anterior sylvian gyrus	<i>MF</i>	medial forebrain bundle
<i>ASG</i>	anterior sigmoid gyrus	<i>MSS</i>	middle suprasylvian gyrus
<i>ASS</i>	anterior suprasylvian gyrus	<i>MSY</i>	middle sylvian gyrus
<i>C</i>	centrum medianum	<i>N</i>	substantia nigra
<i>CL</i>	nucleus centralis lateralis	<i>NVM</i>	nucleus ventromedialis
<i>CM</i>	nucleus centralis medialis	<i>OB</i>	olfactory bulb
<i>CN</i>	nucleus caudatus	<i>ORB</i>	orbital gyrus
<i>COR</i>	coronary gyrus	<i>OS</i>	olfactory stria
<i>DM</i>	nucleus dorsalis medialis	<i>OT</i>	optic tract
<i>F</i>	fornix	<i>P</i>	pes pedunculi
<i>GL</i>	lateral geniculate body	<i>PC</i>	nucleus paracentralis
<i>GM</i>	medial geniculate body	<i>PES</i>	posterior ectosylvian gyrus
<i>GP</i>	globus pallidus	<i>PSG</i>	posterior sigmoid gyrus
<i>GPr</i>	gyrus proreus	<i>PSS</i>	posterior suprasylvian gyrus
<i>H</i>	hypothalamus	<i>PSY</i>	posterior sylvian gyrus
<i>HB</i>	habenula—hab.interped.tract	<i>R</i>	nucleus reticularis
<i>IC</i>	internal capsule	<i>RF</i>	reticular formation—midbrain
<i>IP</i>	inferior thalamic peduncle	<i>S</i>	subthalamus
<i>LA</i>	nucleus lateralis anterior	<i>VA</i>	nucleus ventralis anterior
<i>LAT</i>	gyrus lateralis	<i>VL</i>	nucleus ventralis lateralis
<i>LP</i>	nucleus lateralis posterior	<i>VM</i>	nucleus ventralis medialis
<i>M</i>	mamillary body	<i>VP</i>	nucleus ventralis posterior

CORTICOFUGAL FIBRES TO THE BRAIN-STEM RETICULAR FORMATION. AN EXPERIMENTAL STUDY IN THE CAT

BY GIAN FRANCO ROSSI* AND ALF BRODAL

Anatomical Institute, University of Oslo, Norway

INTRODUCTION

In recent years the reticular formation of the brain stem has been intensively studied by physiological methods. Fairly circumscribed areas have been identified as 'centres' for inhibition and facilitation of cortically and reflexly elicited movements, and the centres have been shown to be subject to influences from the cerebellum, the spinal cord and the cerebral cortex. Respiratory and cardio-vascular 'centres' appear to be closely related topographically to those mentioned above. The demonstration within the brain-stem reticular formation of a 'reticular activating system', exerting its effect on higher levels of the brain, including the cerebral cortex (Moruzzi & Magoun, 1949), adds to the complexity of the functional organization of this part of the central nervous system.

Knowledge of the minute structure of the reticular formation is still insufficient to permit satisfactory correlations with the many new physiological discoveries. The present study represents an attempt to fill one of the gaps in our knowledge of the fibre connexions of the reticular formation. It was undertaken in order to decide whether there are fibres passing from the cerebral cortex directly to those areas of the brain-stem reticular formation in which the 'centres' mentioned have been located by physiological studies.

Several investigators have shown that electrical stimulation of various parts of the cerebral cortex may either inhibit or facilitate functions that are under the influence of the 'centres' of the reticular formation (for references see Kaada, 1951). To what extent these effects are mediated via fibres passing directly from the cerebral cortex to the reticular formation is still a matter of debate. McCulloch, Graf & Magoun (1946), using the strychnine method, have demonstrated a cortico-bulbo-reticular pathway from area 4s, and have brought forward evidence that the impulses, at least in part, follow the course of the pyramidal tract fibres. However, although their experiments are highly suggestive that this is a one-neuron pathway, the findings are not decisive. Also the recording of single-unit activity in the reticular formation following stimulation of the cerebral cortex (Baumgarten, Mollica & Moruzzi, 1954) fails to show decisively if the effects are mediated via direct cortico-bulbar fibres, although the short latencies observed by Amassian & DeVito (1954) and French, Hernández-Péon & Livingston (1955) make this appear likely.

While numerous authors (vide infra) have studied by anatomical methods the efferent fibres descending from the cerebral cortex to the pons, medulla and spinal

* This work was done while Dr Rossi, Physiological Institute, University of Pisa, Italy, was staying in the Anatomical Institute, University of Oslo, with a Norwegian Government grant.

cord, most of them were not interested in the possible termination of cortico-fugal fibres in the reticular formation. Others who have observed fibres of this kind mention them only in passing and give no precise information on where in the reticular formation they end. Since these workers all used the Marchi or cruder methods, comment could not, in any case, have been convincing. However, in the light of present-day knowledge of the functional organization of the brain-stem reticular formation, it is now of interest to know, not only where cortico-reticular fibres take their origin, but also to possess as exact information as possible on their terminal distribution within the reticular formation. This can be achieved only if the fibres degenerating as a consequence of cortical lesions are studied by silver impregnation methods, which allow the tracing of fibres to their ultimate termination.

The present study represents an attempt to investigate the problems of the cortico-reticular projection in this way. It was hoped that it would be possible to determine not only from which cortical regions such fibres take origin and in which areas of the reticular formation they end, but also to see if fibres from different sources have different terminal areas within the reticular formation. Even if decisive answers have not been obtained to all these questions, the results appear to be of sufficient interest to merit publication.

Only the pontine and medullary reticular formation have been studied. The cell groups of the reticular formation projecting on to the cerebellum, namely, the nucleus reticularis tegmenti pontis of Bechterew, the lateral reticular nucleus or nucleus of the lateral funiculus, and the nucleus reticularis paramedianus (Brodal, 1953) will not be considered here.

HISTORICAL

The literature dealing with fibres descending from the cerebral cortex to the brain stem and spinal cord is overwhelming. No attempt has been made to review it completely. Following a scrutiny of a fair proportion of the writings on this subject, those papers have been selected for consideration which make specific reference to fibres passing to the reticular formation. Most of the studies have been made in monkeys, but there are also some relevant observations on human material. In the brief survey to be given below, the data will be grouped according to the origin of cortico-reticular fibres from the four cerebral lobes.

(1) *The frontal lobe*

(a) *Area 4.* Frontal cortico-reticular fibres, more particularly alleged to come from the area 4 of Brodmann, have been described in human material (Marchi method) by Hoche (1898), Dejerine (1901), Sand (1903) and some other authors. Among the first experimental studies relevant to this subject are those of Simpson & Jolly (1907) in the monkey. In Marchi preparations these workers found degenerating fibres leaving the pyramid and entering the contralateral bulbar reticular formation after crossing in the raphe. The fibres are given off along the entire extent of the medullary pyramid. Probst (1899) also described fibres leaving the pyramid and entering the pontine reticular formation. Mettler (1935*b*, 1947), in the monkey, found many fibres from the precentral gyrus passing through the reticular formation to reach the facial, ambiguus and hypoglossal nuclei. Levin (1936) reported similar findings and emphasized the bilateral distribution, with predominance

contralaterally. Verhaart & Kennard (1940) also subscribe to these fibres, but maintain that the cortico-bulbar fibres are very scarce. Minckler, Klemme & Minckler (1944), in a study of human and monkey material with the Marchi and Weigert methods, comment upon the reduction in the number of descending degenerating fibres at levels between the mid-brain and the spinal cord. In Marchi and Weil sections from hemidecorticated rats Combs (1949) describes degenerating fibres passing from the pyramid to the bulbar and pontine tegmentum, and Escolar (1950) in the cat finds degenerating fibres leaving the pyramid and passing to the medial reticular formation of both sides. Finally, Krieg (1954) distinguishes different contingents of fibres, passing from the area 4 to the reticular formation of the lower brain stem.

(b) *Area 4s.* Verhaart & Kennard (1940) deny the existence of fibres from area 4s to levels caudal to the pons, while Marion Hines (1943) finds such fibres in the tegmentum of the bulbar reticular formation. Mettler (1947), while describing fibres to the inferior olive and the hypoglossal nucleus, does not mention fibres to the reticular formation. Ward (1948) claims that fibres from area 4s pass to the caudal pontine reticular formation and from area 24 to its rostral part. All these studies were made by the Marchi method in the monkey.

(c) *Area 6.* A small number of fibres has been traced from this part of the frontal lobe to the bulbar reticular formation by Mettler (1935*b*) and Levin (1936), while Verhaart & Kennard (1940) and Krieg (1954) in the monkey and Escolar (1950) in the cat are inclined to deny their existence.

(2) *The parietal lobe*

Only few reports are available on parieto-reticular fibres. They have been described by Mettler (1935*c*) in the monkey, more particularly from the postcentral gyrus, and in the cat by Escolar (1950). Peele (1942) and Gobbel & Liles (1945) in monkey and cat, respectively, did not observe such fibres.

(3) *The temporal lobe*

Mettler (1935*d*) found temporo-reticular fibres to the medulla, possibly running in the pyramid, in the monkey, while Rundles & Papez (1938) in this animal and Poljak (1927) in the cat deny the existence of temporo-fugal fibres below the pons.

(4) *The occipital lobe*

Mention of fibres to the reticular formation from the occipital lobe is not made by Poljak (1927) and Escolar (1950) studying the occipital fibres in the cat, nor by Mettler (1935*a*) and Nyby & Jansen (1951) in the monkey.

From this review of the literature, although incomplete, it appears that the great majority of cortico-reticular fibres come from the frontal lobe, particularly the 'motor areas', while the existence of such fibres from the other lobes is more equivocal. The available literature on the whole does not give indications that the cortico-reticular fibres are distributed to particular areas or levels of the reticular formation.

MATERIAL AND METHODS

The cats used as experimental animals in the present study are some of those used previously for the study of pyramidal tract fibres from the temporal and occipital lobes by Walberg & Brodal (1953) and for the study of descending fibres to the inferior olive by Walberg (1954, 1955).

Adult cats were subjected to surgical removals of different parts of the cerebral cortex under intraperitoneal nembutal (pentobarbital) anaesthesia. The surgical procedures were

performed under sterile conditions, and, as far as possible, the excisions were limited to the cortex, although usually some of the subcortical white matter was involved in the lesion. Brains of several normal animals served as controls.

The animals were killed 5–8 days after the operation by exsanguination under chloroform or nembutal anaesthesia. The brain and spinal cord were then immediately dissected free and immersed into 10 % formalin for fixation. The area of the cortex containing the lesion was in some of the cases cut away 1 or 2 days later, transferred to 96 % alcohol, embedded in paraffin and cut in serial sections. Every 10th–20th of these was stained with thionine to permit exact identification of the parts damaged by the lesion. In other cases the extent of the lesion was studied in Nissl stained sections from the blocks prepared for silver impregnation (*vide infra*). After fixation for some weeks, the cerebral hemispheres, or what was left of them when the part containing the lesion had been removed, were cut in transverse slices 3–4 mm. thick. From each of these slices several frozen sections 15 μ . thick, were usually cut. The slice itself was preserved so that when the different slices were assembled it was possible to decide exactly which parts of the cortex had been studied.

The brain stems of the cats were cut serially at 15 μ . in horizontal, frozen sections. The sections were collected in groups of fifteen consecutive ones, and of each group two or three were stained according to the Glees (1946) method. In some cases the method of Nauta & Gyax (1951) was employed. The sections were searched for the occurrence of degenerating nerve fibres and terminal boutons.

RESULTS

The normal reticular formation of the brain stem as seen in silver impregnated sections

As is well known the bulbar and pontine reticular formation contains cells of very varying types and sizes, separated by fibre bundles coursing in virtually all directions. Several attempts have been made to subdivide the reticular formation into particular cell groups or nuclei, the most recent and most detailed being the studies of Meessen & Olszewski (1949), Olszewski & Baxter (1954) and Olszewski (1954). Since, however, the borders between the different groups distinguished on a cyto-architectonic basis are in many places somewhat arbitrary, we have chosen to present our findings concerning the distribution of cortico-reticular fibres with reference to drawings of the brain stem only. To what extent there is any correlation with particular nuclei will be considered in the discussion.

For an evaluation of degenerating terminal fibres and boutons in the reticular formation, a knowledge of the normal appearance of these structures is essential. Text-fig. 1 shows in a semidiagrammatic way the types of terminal boutons which may be seen in Glees-impregnated sections from the reticular formation of the normal adult cat's brain stem. As will be seen, there are wide variations. The boutons vary from regular, circular ring-shaped ones, to heavily impregnated, dark and irregular bodies. The most frequent type are the light round or oval rings. Some of the terminal boutons of all types can be seen to be connected with a delicate terminal fibre. Similar variations as in the terminal boutons occur in the so-called *boutons en passage*. From the photomicrographs on Pl. 1, figs. 1–3, it will be seen that the boutons may vary considerably in size. Furthermore, it is common to find boutons of different sizes as well as boutons of different types on the same cell (Pl. 1, figs. 1, 3). Quite frequently, however, the relations of the boutons to nerve cells or their processes cannot be ascertained, since the boutons may be seen lying apparently free in the

spaces between nerve cells and fibres. Whether the wide variations in the appearance of the terminal boutons and *boutons en passage* in normal preparations are due only to technical factors cannot be decided.

Terminal degeneration in the reticular formation and its evaluation

The term 'terminal degeneration' is taken here to denote the degenerative changes occurring in terminal boutons and the terminal fibres leading up to them (or possibly also ending freely), following transection of axons or destruction of their perikarya. The determination of terminal degeneration in the reticular formation meets, however, with greater difficulties than in most other regions of the central nervous system.

It is obvious from the variations in the normal appearance of terminal boutons, described above, that identification of truly degenerating boutons in the reticular

	Terminal boutons		Boutons en passage
	Round or oval	Irregular	
Thin			
Thick			
Solid			
Filamentous			

Text-fig. 1. Drawings of the different types of boutons, terminal and *en passage*, which may be seen in the brain stem reticular formation of the normal cat in Glee sections. The most common types are the round or oval terminal boutons, and among these the thin type is most common, followed by the thick and solid type. The solid irregular boutons may be mistaken for degenerating specimens.

formation will be practically impossible. Some of the types occurring normally resemble very much the picture usually seen when degenerating boutons occur, for example, in the lateral reticular nucleus (Brodal, 1949) or the lateral cervical nucleus (Brodal & Rexed, 1953). As a rule such boutons, in addition to becoming irregular, swell. On account of the normal presence of rather large boutons, size is, however, no reliable criterion, since, for example, a normal large and irregular bouton cannot be distinguished from a degenerating medium-sized one. Only when an unusually large and irregular bouton is seen, may it, with some confidence, be taken as a degenerating specimen of the largest type. To avoid possible errors due to misinterpretations of terminal boutons, we have discarded them in the recordings of changes. Occasionally, an extremely large, compact and irregular bouton has been taken into account.

The exclusion of changes in boutons as criteria of terminal degeneration leaves us with the degenerating fine terminal fibres as evidence of termination of injured cortico-reticular fibres. The changes in the terminal fibres are considered as indications of degeneration when the fibres appear as rows of fine black dots of circular or somewhat irregular shape. Examples are shown in Pl. 1, figs. 5-14.

The structure of the reticular formation makes the recognition of such fine degenerating fibres a difficult and time-consuming procedure. The wealth of fibres of all sizes found everywhere in the reticular formation, even within those areas which have been outlined as particular nuclear groups, gives a background little suited for the identification of fine degenerating fibres. Further, as they course in all directions, only a certain proportion of them, lying approximately in the plane of the sections, can be recognized. In some places, where the cells are more closely grouped, the yellow background is more favourable, but an apparent greater concentration of terminal degenerating fibres in such places may not be real.

Quantitative estimates of degenerating terminal fibres in silver-impregnated preparations are difficult. Only when there are very marked regional differences in their number can comparisons be made on a secure basis. Our experimental animals have been killed 5–8 days after the lesion was made. It is possible that more marked degenerative changes might have been seen with other survival times, but greater differences could scarcely be expected.

Having used the strict criteria outlined above, we feel certain that our findings are reliable. However, it is clear that the changes recorded will represent a minimum of those possibly present. Furthermore, only few quantitative data can be given. The exclusion of degenerating boutons from study also prevents conclusions concerning the mode of termination of the cortico-reticular fibres.

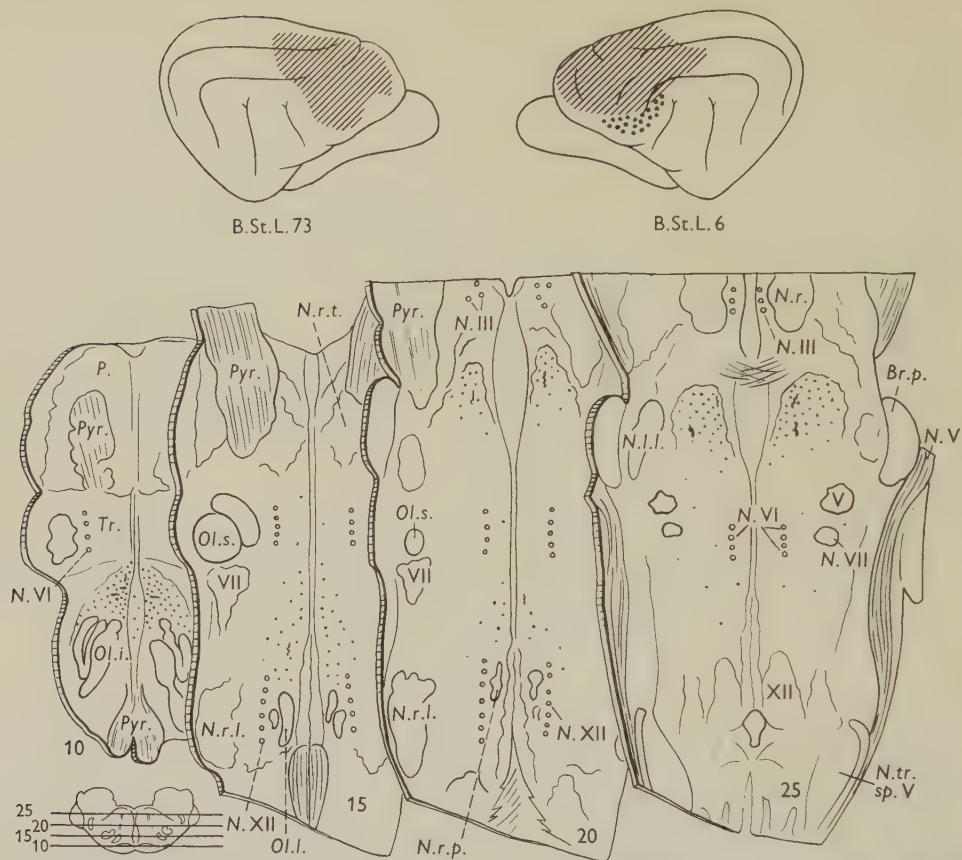
Origin and termination of cortico-reticular fibres

Since the regional distribution of terminal degenerating fibres in the reticular formation was found to be largely identical in cases with lesions in different parts of the cerebral cortex, it will be sufficient to describe their distribution more closely with reference to a few particular experiments. Most intense changes were seen following large fronto-parietal lesions. A case of this type will, therefore, be presented first.

Cat Sp.C.L. 73. Killed after 7 days (Text-fig. 2, and Pl. 1, figs. 4–8). The lesion comprises a considerable part of the cortex in front of and behind the right cruciate sulcus, destroying the entire 'motor area'. In sections through the lesion it is seen that it extends somewhat into the white subcortical matter, but it does not reach the ventricle.

In the horizontal silver-stained sections through the brain stem, scattered degenerating terminal fibres are found almost throughout the reticular formation on both sides, but larger numbers occur only in two fairly circumscribed areas, as shown by the dots in the drawings of Text-fig. 2. On the whole the degeneration is rather scanty also in these areas, and in no place is it of an intensity which can in any way be compared with that seen, for example, in the lateral reticular nucleus following transection of its spinal afferents (Brodal, 1949) or in the gracile and cuneate nuclei following transection of the dorsal columns (Glees & Soler, 1951). Photomicrographs of degenerating terminal and preterminal fibres in this case are shown in Pl. 1, figs. 4–8. On account of the small depth of focus with oil immersion it is seldom possible to get more than one or very few degenerating fibres on the same photograph, but this fact may serve to convey an idea also of the relatively scarce degeneration present. In the drawings of Text-fig. 2 each dot does not represent a certain number of degenerating fibres, but the spacing of dots indicates approximately the relative density of such fibres in different regions.

It will be seen from Text-fig. 2 that of the two regions which contain very many degenerating terminal fibres, most intense degeneration is found in the area of the reticular



Text-fig. 2. Diagram showing the distribution of terminal degenerating fibres in the reticular formation in two cases with large fronto-parietal lesions (cats B.St.L. 73 and 6). The density of the dots gives an impression of the relative number of degenerating terminal fibres in different parts of the reticular formation. Degenerating coarser fibres are shown as wavy lines. Degeneration occurring in other areas than the reticular formation (e.g. the pontine nuclei) is not shown. The numbers of the drawings refer to their order in the series of cat B.St.L. 73. The coarse dots in the diagram of the lesion in cat B.St.L. 6 indicate areas the fibres from which have been damaged.

Abbreviations employed in Text-figs. 2, 4, 6 and 8

<i>B.</i>	Nucleus of Bechterew	<i>N.r.t.</i>	Nucleus reticularis tegmenti pontis of Bechterew
<i>B.c.</i>	Brachium conjunctivum	<i>N.tr.sp.V.</i>	Nucleus of spinal trigeminal tract
<i>Br.p.</i>	Brachium pontis	<i>N. III, V, VI, VII, XII</i>	Root fibres of cranial nerves III, V, VI, VII and XII.
<i>C.i.</i>	Inferior colliculus	<i>Ol.i.</i>	Inferior olive
<i>D.</i>	Nucleus of Deiters	<i>Ol.s.</i>	Superior olive
<i>N.c.e.</i>	External cuneate nucleus	<i>P.</i>	Pontine gray
<i>N.f.c.</i>	Cuneate nucleus	<i>Pyr.</i>	Pyramidal tract fibres
<i>N.f.g.</i>	Gracile nucleus	<i>S.n.</i>	Substantia nigra
<i>N.l.l.</i>	Nucleus of lateral lemniscus	<i>Tr.</i>	Trapezoid body
<i>N.r.</i>	Red nucleus	<i>Tr.sp.V.</i>	Spinal tract of trigeminal nerve
<i>N.r.l.</i>	Lateral reticular nucleus (Nucleus funiculi lateralis)	<i>III, V, VI, VII, XII.</i>	Motor cranial nerve nuclei
<i>N.r.p.</i>	Paramedian reticular nucleus of medulla oblongata		

formation situated immediately dorsal to the rostral half of the inferior olive and a little more rostrally. From this area the degeneration decreases in all directions. The rostral-most medulla shows only slight changes. The same is the case at lower pontine levels, but in the upper pons, dorsal to the pontine nuclei proper, there is an area which is almost as much changed as the area in the lower medulla described above. This pontine area is found caudal to the fibres of the ventral tegmental decussation and is seen in the drawing of sections 20 and 25 in Text-fig. 2. It is also seen in sections farther dorsally, but the degeneration decreases by and by. Ventral to the plane of the abducent nucleus there is, however, still a fair number of degenerating terminal fibres (section 30, not shown in the diagram).

Somewhat coarser, presumably in part preterminal, degenerating fibres are also seen in the same areas. Some of them run horizontally and appear to enter the reticular formation from the raphe, which also contains some degenerating terminal fibres (see Pl. 1, fig. 8). Others have a longitudinal course. The pyramidal tract contains numerous degenerating fibres of different sizes, considerably more on the right than on the left, but owing to the direction of the sections it is not possible to trace degenerating fibres from the pyramids into the reticular formation.

Cat. B.St.L. 6. Killed after 5 days (Text-fig. 2, Pl. 1, fig. 9). The lesion in this case is similar to that in cat B.St.L. 73, described above, but a little more extensive, and encroaches a little on the caudate nucleus. The ensuing terminal degeneration has the same distribution as that mapped in the drawings from the reticular formation in Text-fig. 2, which have been made by means of a projection apparatus from sections in cat B.St.L. 73.

The findings in these two cases show that fibres arising in the fronto-parietal region of the cortex reach the reticular formation of the pons and medulla. The fibres are distributed bilaterally, but whether there is any quantitative difference between the two sides cannot be decided.

In spite of the occurrence of scattered degenerating terminal fibres over almost the entire reticular formation, there are two areas which can clearly be recognized as the chief terminal stations for the cortico-fugal fibres. Thus there is an area of more intense degeneration in the reticular formation immediately dorsal to the rostral half of the inferior olive, and extending from here more rostrally. Another area of more marked changes is found in the rostral pons. This area extends dorsally to a horizontal level passing just in front of the abducent nerve. The changes in the pontine area appear to be a little less intense than in the medullary area. If there are other regional variations, they are too fine to be evaluated.

The lesions in the two cases described above are rather large. However, a considerable proportion of the degenerating fibres in these cases must come from the 'motor area' as outlined by Garol (1942). This is learnt from two cases in which lesions were made in this part of the cortex. Following electro-physiological determination (by stimulation) of the fore-limb and hind-limb area, respectively, these were extirpated (cats B.St.L. 12 and 13). Both animals were killed after 8 days. The lesions are seen in Text-fig. 3. There was only little affection of the underlying white matter.

In the brain-stem reticular formation the distribution of terminal degeneration is essentially as in the animals with larger fronto-parietal lesions, but is somewhat less intense. It is not possible to detect any difference between these two cases with regard to regional distribution of degeneration, which might indicate a somato-topical arrangement of termination of the cortico-reticular fibres.

Lesions in the temporal or occipital lobes are also followed by terminal degenera-

tion in the reticular formation, but the degeneration is much less impressive than in the cases described above. Since the findings are practically similar in the four cases studied only one of them will be described more fully.

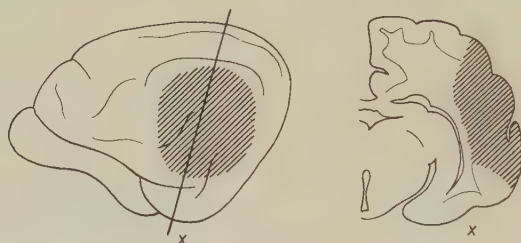
Cat B.St.L. 8. Killed after 8 days (Text-fig. 4, and Pl. 1, fig. 13). The lesion involves the left temporal region, destroying part of the middle and posterior ectosylvian gyri and of the



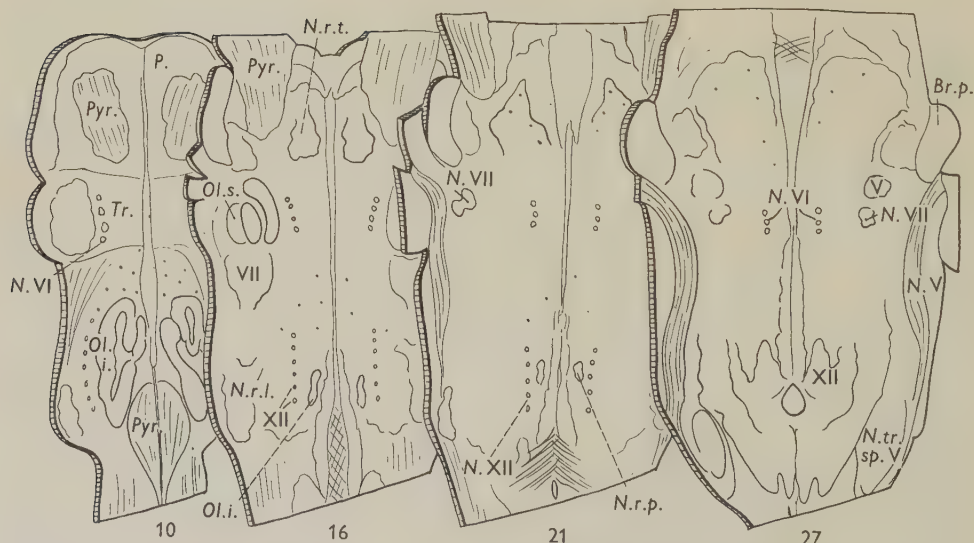
B.St.L. 12

B.St.L. 13

Text-fig. 3. Drawings showing the extent of the lesions in two cases with cortical damage to the motor region (cats B.St.L. 12 and 13). Hatchings indicate parts destroyed, coarse dots areas the fibres from which have been interrupted. The distribution of the ensuing terminal degeneration in the reticular formation is essentially as in the cases shown in Text-fig. 2, but somewhat less marked.



B.St.L. 8

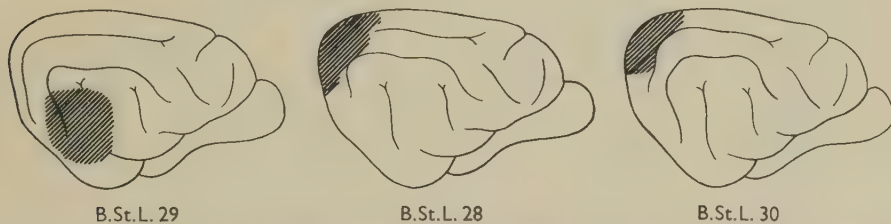


Text-fig. 4. Diagram of the findings in cat B.St.L. 8 after the same principle as in Text-fig. 2. The section to the right (x) shows the depth of the lesion. Abbreviations as in the legend to Text-fig. 2.

anterior sylvian gyrus. In its central, deepest part the lesion approaches the lateral ventricle, but there is a zone of intact white matter left.

In the reticular formation of the brain stem scattered degenerating terminal fibres are seen on both sides, but their number is much smaller than in any of the cases described above. However, as in those cases there is a definite overweight of degeneration in the area dorsal and rostral to the inferior olive, and in the rostral part of the pontine reticular formation. Here a preterminal degenerating fibre is also occasionally seen.

In the cat B.St.L. 29 (killed after 6 days) the temporal lobe lesion (Text-fig. 5) was a little smaller than in the case described above. The distribution of the terminal degeneration in the reticular formation (Pl. 1, figs. 11–12) is, however, the same. Also following lesions of the occipital lobe, comprising chiefly the striate area (cats



Text-fig. 5. Diagram of the lesion in one case with a lesion of the temporal lobe and two cases with lesions of the occipital lobe. The terminal distribution is in all cases essentially as in cat B.St.L. 8, shown in Text-fig. 4.

B.St.L. 28 and 30, both killed after 6 days, Text-fig. 5), the distribution of terminal degeneration is the same. The intensity in all these cases is clearly less than following motor area lesions of a similar size and also less than in cat B.St.L. 8.

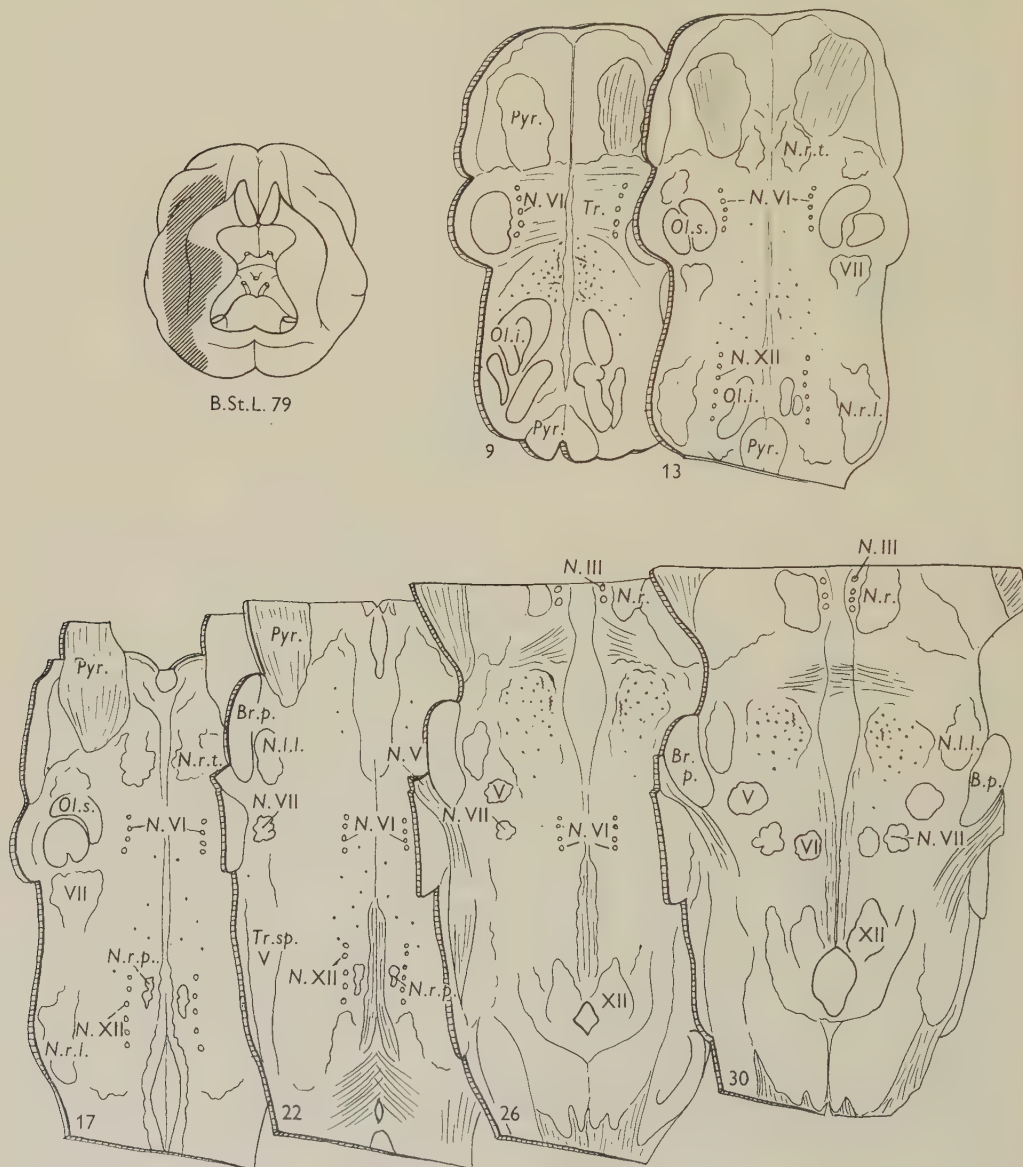
The cases described above demonstrate that there are cortico-reticular fibres from the occipital and temporal lobes. Like those from the fronto-parietal areas they are distributed bilaterally to the pontine and medullary reticular formation, but they are much more scarce. Only in the area dorsal and rostral to the inferior olive and in the upper pons is there some concentration of degenerating terminal fibres.

More important contributions of cortico-reticular fibres come from the basal and medial surfaces of the brain. Text-fig. 6 shows the findings in a relevant case, cat B.St.L. 79 (killed after 7 days). While a few terminal degenerating fibres are found scattered throughout the reticular formation, larger numbers occur bilaterally in the same pontine and medullary areas which show most changes following lesions of other parts of the cortex.

In cat B.St.L. 79 (Text-fig. 6) the lesion of the basal surface was rather extensive, and included parts of the orbital surface and posteriorly the greater part of the pyriform lobe, as well as smaller parts of the lateral and posterior suprasylvian gyri. In another animal with a basal lesion, cat B.St.L. 48 (Text-fig. 7), a thermocautery lesion damaged the right orbital and anterior ectosylvian gyri, and in a third (cat B.St.L. 75) the lateral part of the pyriform lobe and adjacent parts of the anterior and posterior sylvian gyri were damaged. In both cases the distribution of terminal degeneration is as in cat B.St.L. 79, but the degeneration is considerably less intense. Since the lesion in cat B.St.L. 75 involves parts of the pyriform lobe as well as neo-

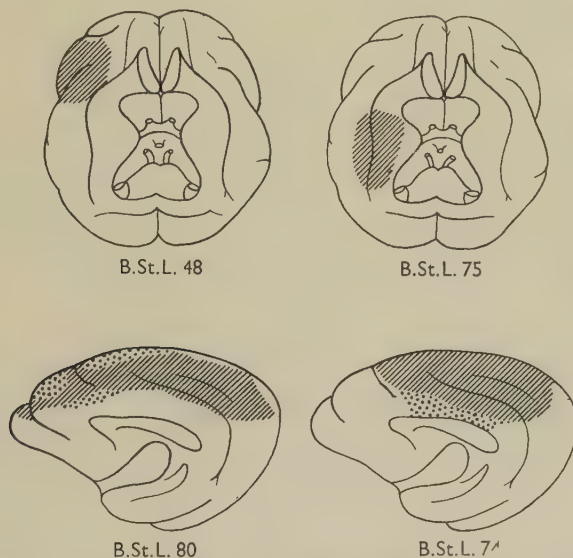
cortical parts it is not possible to decide whether the lesion of the palaeo-cortex is responsible for the terminal degeneration. However, it can at least be concluded that fibres to the reticular formation take origin from anterior as well as posterior regions of the basal cortex of the cerebrum.

In Text-fig. 7 are also shown the lesions in two cats, in which the medial cortex was damaged. In both cases the distribution of the terminal degeneration is similar



Text-fig. 6. Diagram of the findings in cat B.St.L. 79, having an extensive lesion of the basal surface. The distribution of terminal degeneration is shown as in the diagram in Text-fig. 2.

to that found following lesions of other parts of the cortex. The intensity of degeneration is almost the same as that following a large basal lesion (cat B.St.L. 79, Text-fig. 6). In cat B.St.L. 80 the lesion extends so far in an anterior direction that it has destroyed the cortex surrounding the cruciate sulcus, i.e. presumably part of the cortex belonging to the 'motor area'. Since, however, degeneration is not more, but rather less, marked in this case than in cat B.St.L. 74, it may be assumed that cortico-reticular fibres take origin from the medial surface posterior to the cruciate sulcus, i.e. from the cingulate and/or the splenial and suprasplenial gyri.



Text-fig. 7. Diagram showing the lesions in four animals, B.St.L. 48, 75, 80 and 74—killed after 6, 7, 6, and 7 days respectively. Coarse dots indicate areas the fibres from which have been cut. Terminal degeneration in all cases in the same areas as shown for cat B.St.L. 79 in Text-fig. 6. The intensity of degeneration is described in the text.

It appears from our findings that cortico-fugal fibres from all regions of the cortex studied have two common terminal areas in the reticular formation, one in the pons, another in the medulla.* In Text-fig. 8 these two chief terminal areas are shown as projected on a series of sagittal drawings. For comparison the distribution of reticular neurons having long ascending axons (Brodal & Rossi, 1955) is also shown. It will be seen that there is relatively little overlap. The possible significance of this will be considered in the discussion.

* It should again be emphasized that in this study we have left out of account those parts of the reticular formation which project on to the cerebellum. To avoid misunderstandings, however, it may be mentioned that cortical fibres reach also these nuclei (the nucleus reticularis tegmenti pontis of Bechterew, the nucleus reticularis lateralis or funiculi lateralis, and the nucleus reticularis paramedianus medullae oblongatae). A detailed analysis of these subdivisions of the cortico-reticular projection remains to be done.

DISCUSSION

As mentioned above, degenerating terminal boutons in the reticular formation cannot always be distinguished from normal ones, since the latter show wide normal variations (see Text-fig. 1). We have, therefore, restricted our recordings of degenerative changes to those occurring in the terminal fibres. Their characteristic appearance in the experimental animals and the absence of similar fibres in normal controls leave no doubt that we are dealing with truly degenerating terminal and preterminal fibres.



Text-fig. 8. A series of sagittal sections through the cat's brain stem, showing the chief terminal areas of cortico-reticular fibres (dots) as determined in the present study and the sites of reticular neurons having long ascending axons (vertical bars) as determined previously (Brodal & Rossi, 1955).

It is clear from our findings that the number of fibres in the reticular formation of the cat, degenerating as a consequence of cortical lesions, is not very large. This agrees with the opinions expressed by Verhaart & Kennard (1940) and others on the basis of Marchi studies in the monkey. However, different parts of the cerebral cortex send varying numbers of fibres to the reticular formation of the brain stem. Since most lesions were not limited to particular cytoarchitectonic areas, the origin of the fibres cannot be given with reference to specific areas except for the 'motor area'. This, without doubt, is an important source of cortico-reticular fibres and appears to give origin to a great proportion of all fibres from the fronto-parietal cortex to the reticular formation. Basal areas of the brain, as well as parts of the medial surface, also contribute considerably to the cortico-reticular projection. In experimental lesions involving the sensory acoustic and particularly the optic areas, on the other hand, very little degeneration of cortico-reticular fibres is found.

The relative contributions determined here agree fairly well with the findings made in Marchi material by previous students, particularly concerning the predominance of fronto-reticular fibres (see p. 43). Our findings make clear that the disputed fibres from the temporal and occipital lobes do exist, even if their number is very modest. That such fibres may be more abundant to the mesencephalic reticular formation may be surmised, since, by physiological methods, Jasper, Ajmone-Marsan & Stoll (1952), Bremer & Terzuolo (1954) and French *et al.* (1955) found evidence for ample projections to this region from various parts of the cerebral cortex. Further, and in agreement with our anatomical findings, Scheibel, Scheibel, Mollica & Moruzzi (1955) report that electrical or strychnine stimulation of the acoustic cortex was quite ineffective in producing micro-electrode responses from the lower medulla. Our findings confirm Ward's (1948) concerning fibres from area 24 to the pontine reticular formation and to the medulla oblongata.

It is interesting to note that with regard to their sites of origin the cortico-reticular fibres show a parallelism with certain other cortico-fugal systems, for example, the pyramidal tract. In addition to fibres from the 'motor' and 'premotor' areas, this tract contains smaller contingents from the occipital and temporal lobes (Walberg & Brodal, 1953), and it appears that there are also cortico-spinal fibres from the medial and basal surface of the cerebrum (unpublished observations). There is also a correspondence between the sources of cortical fibres to the inferior olive (Walberg, 1954, 1955) and to the reticular formation.

The method used in this study is not well suited to determine the course taken by the cortico-reticular fibres. If they descend intermingled with, or are collaterals from, the fibres of the pyramidal tract, as appears likely (see Cajal, 1909), they might be recognized where they take off from the main bundle. Although we have frequently seen degenerating preterminal fibres in the changed areas of the reticular formation, coursing longitudinally or transversely, we have never been able to follow fibres of this type from the bundles of the pyramidal tract. This inability may in part be due to the direction of sectioning which is little favourable for such study. However, our findings do not contradict the observations of several previous authors that the cortico-reticular fibres descend among those of the pyramidal tract or are collaterals from such fibres.

The advantage of the method employed in this study is that it makes it possible to establish the areas of termination of the cortico-reticular fibres, a problem which could not be solved by the Marchi method used by previous authors. As has been seen, fibres from widely different parts of the cerebral cortex terminate in identical areas of the reticular formation. These areas, therefore, may be considered as sites of convergence of impulses from various cortical regions. That convergence of this type actually occurs in the reticular formation has been shown by Bremer & Terzuolo (1954) and Scheibel *et al.* (1955). The findings concerning the fibres from the motor area, furthermore, indicate that there is no somatotopical subdivision within the terminal areas. However, these conclusions must be made with certain qualifications, since it is impossible to make exact quantitative estimates of degenerating terminal fibres. For this reason there may be within the total terminal areas determined in our experiments, smaller regional differences which betray a certain differential distribution, but which have escaped recognition. For the same reason

our finding of an approximately equal number of degenerating fibres on both sides of the reticular formation, following a unilateral cerebral lesion, probably requires qualifications.

Although scattered cortico-reticular fibres appear to reach almost all parts of the pontine and medullary reticular formation, it is clear from our findings that the bulk of the fibres reach two terminal areas, one in the medulla, another in the pons. Their situation is seen in Text-figs. 2, 4, 6 and 8. They are separated by areas of the reticular formation receiving very few or practically no cortico-fugal fibres. The two areas appear to be almost equally important with regard to the number of fibres they receive.

The demonstration of two fairly well circumscribed terminal areas for the cortico-reticular fibres raises the question whether these areas correspond to definite structurally characterized parts of the reticular formation, and whether there is any correlation between them and the areas outlined by physiological methods as 'centres' for different functions. A comparison with Meessen & Olszewski's (1949) atlas of the rabbit's brain stem shows that the pontine area determined here as a terminal station for cortico-reticular fibres coincides almost completely with the part of the reticular formation outlined by these authors as the 'nucleus pontis oralis' which can be recognized also in the horizontal silver sections. Degeneration appears to be a little more marked in its rostral part than caudally, but there is also clear-cut but scanty degeneration in the rostral-most part of the nucleus reticularis pontis caudalis. The medullary terminal area corresponds to Meessen & Olszewski's 'nucleus gigantocellularis medullae oblongata', but the dorsal regions of this are certainly less changed than the ventral parts, situated just dorsal to the inferior olive. Furthermore, it appears that degeneration is more abundant in the rostral part of the nucleus than in the caudal.

These findings tend to support the assumption that the subdivisions of the brain-stem reticular formation which can be made on a cytoarchitectonic basis have functional significance. For an understanding of the functional importance of the cortico-reticular fibres, it is necessary to know the destination of the efferent fibres from their two terminal areas. It appears from preliminary studies of the origin of descending fibres from the reticular formation (unpublished) that following transection of the spinal cord at high cervical levels, large numbers of altered nerve cells are found in those parts of the reticular formation which according to the present study are the chief end-stations for the cortico-reticular fibres. The medullary terminal area appears to correspond also to the area determined by Pitts (1940) as the site of origin of reticulo-spinal fibres. Finally, Papez (1926) in his Marchi experiments found evidence that reticulo-spinal fibres from the medulla come from 'large reticular cells of the upper oblongata' and from 'large reticular elements of the pons and isthmus'. When taken together the data given here thus make it appear likely that the cortico-reticular fibres terminate chiefly in areas of the reticular formation, from which a large proportion of reticulo-spinal fibres take origin, i.e. that the cortico-reticular projection system is a link in pathways capable of transmitting impulses of cortical origin to cells in the spinal cord.

It would, however, be an undue over-simplification to assume that this should be their only task. A comparison of the terminal areas of the cortico-reticular fibres

with the regional distribution of reticular neurons sending their axons in a rostral direction is illuminating in this respect. In a recent study (Brodal & Rossi, 1955) it has been shown that scattered cells sending their axon rostrally beyond the mesencephalon occur over large areas of the reticular formation, but they are collected in greater numbers in certain places only. One of these areas is that surrounding the root fibres of the abducent nerve, corresponding to a large part of Meessen & Olszewski's 'nucleus reticularis pontis caudalis'. Another such area is found in the medulla dorsal to the inferior olive and comprises most of the nucleus reticularis gigantocellularis, but also the ventral parts of the nucleus reticularis lateralis of Meessen & Olszewski and the medial parts of their ventral reticular nucleus. (In addition, a certain number of ascending fibres take origin in the mesencephalon.) From Text-fig. 8 where the distribution of rostrally projecting cells is shown as contrasted with the sites of termination of cortico-reticular fibres, it will be seen that the majority of ascending reticular fibres take origin from other parts of the reticular formation than those in which the cortico-reticular fibres terminate. This is particularly evident in the pons. However, dorsal to the inferior olive, i.e. in the nucleus reticularis gigantocellularis, there is a common area (even if the rostral part of this nucleus contains few ascending neurons but many terminations of cortico-fugal fibres, and the adjoining areas, rich in rostrally projecting cells are almost free from terminations of cortico-reticular fibres). Thus we have here apparently a mechanism whereby impulses from the cerebral cortex may influence the activity of the rostrally projecting reticular system.*

It is remarkable that with the exception of the region just mentioned, the areas of the reticular formation particularly rich in rostrally projecting neurons, are other than those receiving cortico-reticular fibres (and probably containing chiefly caudally projecting neurons, *vide supra*). The findings so far available, therefore, cast considerable doubt on the common assumption that the brain-stem reticular formation is entirely diffusely organized. However, the segregation into regions having different fibre connexions and presumably different functions is certainly not sharp, as appears from the termination of scattered cortico-reticular fibres outside their chief terminal areas and from the wide distribution of neurons projecting rostrally. Other features point in the same direction, as, for example, the presence within the reticular formation of numerous cells having an axon which dichotomizes into an ascending and a descending branch.

It is a question of considerable interest whether the cortico-reticular fibres establish synaptic contact with cells of all types or with cells of one or a few types only. Since we have left out of consideration the alterations occurring in the terminal boutons in our experimental animals, no answer can be given to this question.

* That this is not a unique example of cortico-fugal fibres passing to rostrally projecting nuclei is learnt from our observation (not mentioned in the descriptions given above) that a fair number of cortico-fugal fibres terminate in the spinal trigeminal nucleus and the nucleus of the solitary tract. There appears to be also some degeneration in the immediately adjoining areas of the reticular formation, but with the direction of sectioning used, this is difficult to decide. These anatomical findings are of particular interest in connexion with Hagbarth & Kerr's (1954) recent physiological observations of central inhibitory influences on afferent conduction in the spinal cord.

Functional correlations

While concerned primarily with structural features the considerations made above also have important bearings on problems of the physiology of the reticular formation. It would take us too far to discuss the anatomico-physiological correlations fully, but it will be appropriate to mention some relevant points.

As is well known, respiratory movements, cardio-vascular functions and the activity of skeletal muscles can be influenced by stimulation of various parts of the cerebral cortex. However, concerning the pathways which mediate these effects there is still considerable doubt. While there is evidence that some of them may be mediated at least in part through pathways involving various subcortical structures, it is possible that a certain role is also played by direct corticofugal fibres to 'centres' of the brain stem which have been identified as subserving the regulation of the functions in question. The observations of McCulloch *et al.* (1946), Ward (1948) and McCulloch and Henneman (1948) who recorded spikes in the reticular formation following strychninization of areas 4s, 24 and 19, respectively, have been taken to support this view, as have also the single unit recordings of Baumgarten *et al.* (1954).

In discussing whether our findings are in agreement with this concept, it is essential to consider whether the terminal areas of cortico-reticular fibres determined here coincide with those parts of the reticular formation which have been found by physiological methods to subserve the various functions. Such a comparison, however, meets with certain difficulties. Points and areas stimulated or recorded from are not always given with sufficiently detailed reference to anatomical features. Furthermore, there is always the possibility that the structures stimulated or recorded from have been not perikarya but afferent or efferent fibres of the structures in question. In spite of these factors of incertitude a comparison shows that, on many points, there is a remarkable correspondence between the anatomical and physiological findings.

Thus the cortico-fugal terminal area in the nucleus reticularis giganto-cellularis of the medulla (see Text-figs. 2, 4, 6 and 8) covers most of the field indicated by Maguon & Rhines (1946) and also by later authors as the area harbouring an inhibitory mechanism for motor activities. The cardio-vascular depressor centre of Alexander (1946) appears to be almost coincident with the same area as does also the inspiratory centre of Pitts (1940). The cell group labelled NR IV in Pitts's drawings appears to be the nucleus reticularis gigantocellularis of Meessen & Olszewski (1949).

The spatial correspondence between the terminal area of cortico-reticular fibres and the area allotted to the various 'centres' in the medulla seems to be sufficiently close to warrant the conclusion that the cortico-reticular fibre system described here may be concerned in the inhibition of muscular activity, inspiratory effects and cardio-vascular depressor effects obtainable from stimulation of various parts of the cortex. The fact that the areas in the medulla subserving these diverse functions appear to be practically the same is paralleled by the fact that all three types of effects may be obtained from some of the regions considered here (see Kaada, 1951). Furthermore, it is of interest that effects of one type or the other have been obtained

from stimulation of all regions shown to project on to the medullary reticular formation.

Concerning the pontine cortico-reticular terminal area correlation with the physiological findings is less clear. It appears, however, that this area (comprising chiefly the nucleus pontis oralis) is part of the region of the brain-stem reticular formation shown by Rhines & Magoun (1946) and others to exert a facilitatory effect on motor activities, even if this area appears to extend also a little more caudally, as well as rostrally, into the mesencephalon (the possible cortico-reticular fibres to this have, however, not been included in our study). Pitts's (1940) expiratory centre, however, seems to be limited to the dorsal parts of the medullary reticular formation, and Alexander's (1946) pressor centre appears to be situated largely caudal to the chief cortico-reticular terminal area in the pons. However, this may be due to the fact that, in part, descending fibres as well as nerve cells have been stimulated in the physiological experiments.

On the whole, it appears likely that the cortico-fugal fibres to the pontine reticular formation (and possibly to the mesencephalic as well) may be utilized by impulses from the cerebral cortex responsible for motor facilitation, while their possible role for cardio-vascular pressor and respiratory effects seems more equivocal. It is in agreement with the widespread origin of the cortico-reticular fibres that the facilitatory effects supposed to be mediated via the reticular centres may be obtained under experimental conditions from various parts of the cortex (see Kaada, 1951).

As mentioned above, reticular cells sending their axons in a rostral direction and passing beyond the mesencephalon are present within the medullary terminal area of cortico-reticular fibres. Cortical fibres to this area, therefore, may be capable of influencing the activity in the 'reticular activating system' as suggested by Kaada (1951, p. 240). Jasper, Ajmone-Marsan & Stoll (1952), Bremer & Terzuolo (1954), and French *et al.* (1955) have brought forward electro-physiological evidence for cortico-fugal projections from different parts of the cerebral cortex to the mesencephalic reticular formation which contains a fair number of rostrally projecting neurons (Brodal & Rossi, 1955). Bremer & Terzuolo, who have shown that stimulation of the cerebral cortex alters the activity in the ascending activating system, discuss the role which such cortico-reticular connexions may play in the mechanism of sleep and awakening, and French *et al.* point to their importance for the maintenance of attention and introspection and their possible capacity of modulating sensory inputs to the brain. Similar considerations might presumably also be applied to the medullary area which in part projects rostrally and receives cortico-fugal fibres.

SUMMARY

Lesions have been made in different parts of the cerebral cortex of adult cats, and the reticular formation of the pons and medulla have been searched in horizontal silver-impregnated sections (Glees's method) for the occurrence of signs of terminal degeneration.

Since terminal boutons in normal animals may show variations which resemble degenerating terminal boutons, only characteristic degenerating terminal fibres have been taken into account as evidence of terminal degeneration.

Fibres to the pontine and medullary reticular formation appear to take origin from all parts of the cerebral cortex. The 'motor area' is the most important source, but substantial contributions come also from the basal and medial surfaces. Only few fibres come from the temporal and occipital lobes. The fibres probably descend among those of the pyramidal tract.

Regardless of their source within the cortex the fibres are distributed to identical parts of the reticular formation. Scattered fibres may terminate all over the reticular formation, but the majority of them end in two fairly well-circumscribed areas, a pontine region corresponding to Meessen & Olszewski's (1949) nucleus pontis oralis, and a medullary area, corresponding to their nucleus reticularis gigantocellularis. The cortico-reticular fibres appear to be distributed in approximately equal numbers to both halves of the brain stem, but since quantitative estimates are difficult in silver-impregnated sections this statement may require qualifications.

Functional implications of the findings are briefly discussed. It is concluded that the organization of the cortico-reticular fibres demonstrated is compatible with the assumption that this fibre system is engaged in the transmission to the brain stem reticular formation of impulses mediating cortical effects on somato-motor activity, respiration and cardio-vascular functions. The fibres may also influence activity in the ascending activating system.

REFERENCES

- ALEXANDER, R. S. (1946). Tonic and reflex functions of medullary sympathetic cardiovascular centers. *J. Neurophysiol.* **9**, 205–217.
- AMASSIAN, V. E. & DEVITO, R. (1954). Unit activity in reticular formation and nearby structures. *J. Neurophysiol.* **17**, 575–603.
- BAUMGARTEN, R. V., MOLICA, A. & MORUZZI, G. (1954). Modulierung der Entladungsfrequenz einzelner Zellen der Substantia reticularis durch corticofugale und cerebelläre Impulse. *Pflüg. Arch. ges. Physiol.* **259**, 56–78.
- BREMER, F. & TERZUOLO, C. (1954). Contribution à l'étude des mécanismes physiologiques du maintien de l'activité vigile du cerveau. Interaction de la formation réticulée et de l'écorce cérébrale dans le processus du réveil. *Arch. int. Physiol.* **62**, 157–178.
- BRODAL, A. (1949). Spinal afferents to the lateral reticular nucleus of the medulla oblongata in the cat. An experimental study. *J. comp. Neurol.* **91**, 259–295.
- BRODAL, A. (1953). Reticulo-cerebellar connections in the cat. An experimental study. *J. comp. Neurol.* **98**, 113–153.
- BRODAL, A. & REXED, B. (1953). Spinal afferents to the lateral cervical nucleus in the cat. An experimental study. *J. comp. Neurol.* **98**, 179–212.
- BRODAL, A. & ROSSI, G. F. (1955). Ascending fibers in brain stem reticular formation of cat. *A.M.A. Arch. Neurol. Psychiat.* **74**, 68–87.
- CAJAL, S. R. (1909). *Histologie du Système Nerveux de l'Homme et des Vertébrés*. Paris: Maloine.
- COMBS, C. M. (1949). Fiber and cell degeneration in the albino rat brain after hemidecortication. *J. comp. Neurol.* **90**, 373–401.
- DEJERINE, J. (1901). *Anatomie des Centres Nerveux*. Paris.
- ESCOLAR GARCÍA, J. (1950). Aportaciones al estudio de las vías piramidales y extrapiramidales. Campos corticales de proyección centrifuga en el gato por el método de Marchi y Rasdolsky. *Act. Med.* **36**, 343–348.
- FRENCH, J. D., HERNÁNDEZ-PÉON, R. & LIVINGSTON, R. B. (1955). Projections from cortex to cephalic brain stem (reticular formation) in monkey. *J. Neurophysiol.* **18**, 74–95.
- GAROL, H. W. (1942). The 'motor' cortex of the cat. *J. Neuropath.* **1**, 139–145.
- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath.* **5**, 54–59.

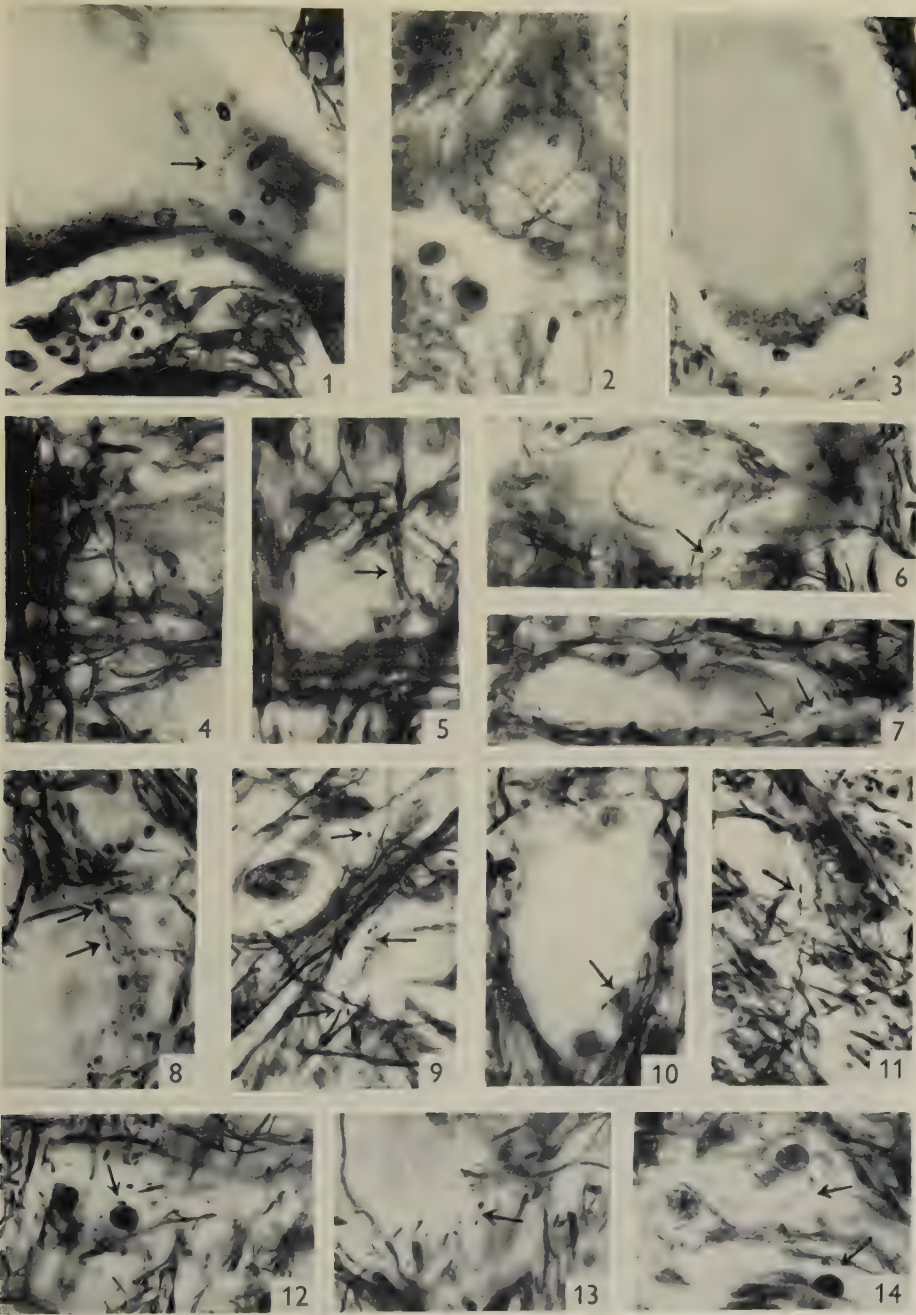
- GLEES, P. & SOLER, J. (1951). Fibre content of the posterior column and synaptic connections of nucleus gracilis. *Z. Zellforsch.* **36**, 381–400.
- GOBBEL, W. G. JR. & LILES, G. W. (1945). Efferent fibers of the parietal lobe of the cat (*Felis domesticus*). *J. Neurophysiol.* **8**, 257–266.
- HAGBARTH, K.-E. & KERR, D. I. B. (1954). Central influences on spinal afferent conduction. *J. Neurophysiol.* **17**, 295–307.
- HINES, M. (1943). Control of movements by cerebral cortex in primates. *Biol. Rev.* **18**, 1–31.
- HOCHE, A. (1898). Beiträge zur Anatomie der Pyramidenbahn und der oberen Schleife, nebst Bemerkungen über die abnormen Bündel in Pons und Medulla oblongata. *Arch. Psychiat.* **30**, 103–136.
- JASPER, H., AJMONE-MARSAN, C. & STOLL, J. (1952). Cortico-fugal projections to the brain stem. *A.M.A. Arch. Neurol. Psychiat.* **67**, 155–171.
- KAADA, B. R. (1951). Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of 'rhinencephalic' and other structures in primates, cat and dog. *Acta physiol. scand.* **24** (Suppl. 83), 1–285.
- KRIEG, W. J. S. (1954). *Connections of the Frontal Cortex of the Monkey*. Springfield: Charles C. Thomas.
- LEVIN, P. M. (1936). The efferent fibers of the frontal lobe of the monkey, *Macaca mulatta*. *J. comp. Neurol.* **63**, 369–419.
- MCCULLOCH, W. S., GRAF, C. & MAGOUN, H. W. (1946). A cortico-bulbo-reticular pathway from area 4s. *J. Neurophysiol.* **9**, 127–132.
- MCCULLOCH, W. S. & HENNEMAN, E. (1948). The projection of area 19 to the reticular formation. *Fed. Proc.* **7**, p. 79.
- MAGOUN, H. W. & RHINES, R. (1946). An inhibitory mechanism in the bulbar reticular formation. *J. Neurophysiol.* **9**, 165–171.
- MEESSEN, H. & OLSZEWSKI, J. (1949). *A Cytoarchitectonic Atlas of the Rhombencephalon of the Rabbit*. Basel, New York: S. Karger.
- METTLER, F. A. (1935a). Corticofugal fiber connections of the cortex of *Macaca mulatta*. The occipital region. *J. comp. Neurol.* **61**, 221–256.
- METTLER, F. A. (1935b). Corticofugal fiber connections of the cortex of *Macaca mulatta*. The frontal region. *J. comp. Neurol.* **61**, 509–542.
- METTLER, F. A. (1935c). Corticofugal fiber connections of the cortex of *Macaca mulatta*. The parietal region. *J. comp. Neurol.* **62**, 263–292.
- METTLER, F. A. (1935d). Corticofugal fiber connections of the cortex of *Macaca mulatta*. The temporal region. *J. comp. Neurol.* **63**, 25–48.
- METTLER, F. A. (1947). Extracortical connections of the primate frontal cerebral cortex. II. Corticofugal connections. *J. comp. Neurol.* **86**, 119–166.
- MINCKLER, J., KLEMME, R. M. & MINCKLER, D. (1944). The course of efferent fibers from the human premotor cortex. *J. comp. Neurol.* **81**, 259–267.
- MORUZZI, G. & MAGOUN, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Electroenceph. clin. Neurophysiol.* **1**, 455–473.
- NAUTA, W. J. H. & GYGAX, P. A. (1951). Silver impregnation of degenerating axon terminals in the central nervous system: (1) Technic. (2) Chemical notes. *Stain Tech.* **26**, 5–11.
- NYBY, O. & JANSEN, J. (1951). An experimental investigation of the cortico-pontine projection in *macaca mulatta*. *Norske Vid. Akad., Avh. I, Math.-Naturv. Kl.*, no. 3, pp. 1–47.
- OLSZEWSKI, J. (1954). The cytoarchitecture of the human reticular formation. In *Brain Mechanisms and Consciousness*, pp. 54–80. Oxford: Blackwell.
- OLSZEWSKI, J. & BAXTER, D. (1954). *Cytoarchitecture of the Human Brain Stem*. New York, Basel: S. Karger.
- PAPEZ, J. W. (1926). Reticulo-spinal tracts in the cat. *J. comp. Neurol.* **41**, 365–399.
- PEELE, T. L. (1942). Cytoarchitecture of individual parietal areas in the monkey (*Macaca mulatta*) and the distribution of the efferent fibers. *J. comp. Neurol.* **77**, 693–737.
- PITTS, R. F. (1940). The respiratory center and its descending pathways. *J. comp. Neurol.* **72**, 605–625.
- POLJAK, S. (1927). An experimental study of the association callosal, and projection fibers of the cerebral cortex of the cat. *J. comp. Neurol.* **44**, 197–258.
- PROBST, M. (1899). Zur Kenntnis der Pyramidenbahn (normale und abnormale Pyramidenbündel und Reizversuche der Kleinhirnrinde). *Msschr. Psychiat. Neurol.* **6**, 91–113.

- RHINES, R. & MAGOUN, H. W. (1946). Brain stem facilitation of cortical motor response. *J. Neurophysiol.* **9**, 219–229.
- RUNDLES, R. W. & PAPEZ, J. W. (1938). Fiber and cellular degeneration following temporal lobectomy in the monkey. *J. comp. Neurol.* **68**, 267–296.
- SAND, R. (1903). Beitrag zur Kenntnis der cortico-bulbaren und cortico-pontinen Pyramidenfasern beim Menschen. *Arch. neurol. Inst. (Inst. Anat. Physiol. Zentr.Nerv.) Univ. Wien*, **10**, 185–222.
- SCHEIBEL, M., SCHEIBEL, A., MOLICA, A. & MORUZZI, G. (1955). Convergence and interaction of afferent impulses on single units of reticular formation. *J. Neurophysiol.* **18**, 309–331.
- SIMPSON, S. & JOLLY, W. A. (1907). Degenerations following experimental lesions in the motor cortex of the monkey. *Proc. R. Soc. Edinb.* **27**, 281–301.
- VERHAART, W. J. C. & KENNARD, M. A. (1940). Corticofugal degeneration following thermocoagulation of areas 4, 6, and 4s in *Macaca mulatta*. *J. Anat., Lond.*, **74**, 239–254.
- WALBERG, F. (1954). Descending connections to the inferior olive. In Jansen & Brodal, *Aspects of Cerebellar Anatomy*, pp. 249–263. Oslo: Johan Grundt Tanum.
- WALBERG, F. (1955). Descending connections to the inferior olive. An experimental study in the cat. *J. comp. Neurol.* (in the Press).
- WALBERG, F. & BRODAL, A. (1953). Pyramidal tract fibers from temporal and occipital lobes. An experimental study in the cat. *Brain*, **76**, 491–508.
- WARD, A. A. JR. (1948). The cingular gyrus: area 24. *J. Neurophysiol.* **11**, 13–24.

EXPLANATION OF PLATE

Photomicrographs from the brain stem reticular formation. Gleys method. $\times 850$.

- Figs. 1–3. From the normal cat, showing terminal boutons of different sizes and types on the same cell (fig. 1), boutons *en passage* (fig. 2) and solid irregular boutons (fig. 3).
- Figs. 4–8. From cat B.St.L. 73, having a large fronto-parietal lesion (see Text-fig. 2). Terminal and preterminal degenerating fibres (arrows) are seen, figs. 4–7 from the medullary reticular formation, fig. 8 from the raphe of the medulla.
- Fig. 9. From cat B.St.L. 6, showing preterminal and terminal degenerating fibres in the pontine reticular formation following a large fronto-parietal lesion.
- Fig. 10. From the medial bulbar reticular formation in cat B.St.L. 12, having a lesion of the motor area (see Text-fig. 3).
- Figs. 11–12. From the bulbar (fig. 11) and pontine (fig. 12) reticular formation in cat B.St.L. 29, having a lesion of the temporal lobe (see Text-fig. 5).
- Fig. 13. Fragments of a fine terminal degenerating fibre in the medullary reticular formation of cat B.St.L. 8, having a temporal lesion (see Text-fig. 4).
- Fig. 14. From the pontine reticular formation in cat B.St.L. 79, having a lesion of the basal surface (see Text-fig. 6).



ROSSI AND BRODAL—CORTICO-RETICULAR FIBRES

(Facing p. 62)

THE GROWTH OF THE FOETAL SKULL

By E. H. R. FORD

Department of Anatomy, St Thomas's Hospital Medical School

INTRODUCTION

Quantitative work on the growth of the foetal skull has hitherto been confined to studies of the undissected foetal head. The dried foetal skull is of limited value owing to the shrinkage and distortion produced by drying. The most important work on the growth of the foetal head is that of Scammon & Calkins (1929), who summarize the previous work on the subject. Their findings are that most dimensions of the foetal head can be related to crown-heel length of the foetus by the formula $HD = aL + b$, where HD is any given diameter of the head, L is crown-heel length, and a and b constants differing for each diameter. The only exceptions are the bimalar and orbito-auricular diameters. The cranium becomes longer and broader in relation to its height; the face grows relatively more in height and breadth than the cranium, and thus becomes in comparison broader. The various cranial dimensions increase five- to sevenfold from 3 months to birth.

Since all their measurements are external, Scammon & Calkins are unable to analyse differential growth rates within the skull. In the present study, by analysing the growth of different parts of the foetal skull, an attempt is made to explain the changes in form which result from differential growth rates within it.

MATERIAL AND METHODS

Measurements have been made on seventy-six foetuses of menstrual ages between 10 and 40 weeks, the majority of which have been preserved in formalin, the remainder (seven) being fresh. Exact histories not being usually available, ages have been assigned as follows: crown-rump length, foot length, and weight have been recorded where possible, and then the age has been assigned by means of Streeter's (1920) tables. Where there is a discrepancy between the three measurements, crown-rump length has been taken as the most reliable, and the history (when available) has also been taken into consideration. For ages between 10 and 22 weeks the foetuses have then been arranged in weekly groups (the 10-week group containing all foetuses of estimated age 10 weeks or over but less than 11 weeks) and for ages between 22 and 40 weeks in two-weekly groups (the 22-week group containing all foetuses of estimated age 22 weeks or over but less than 24 weeks). The average of all measurements of a dimension within one group has then been given as the mean for that group.

All linear measurements have been made with dividers and a millimetre rule, measurements being recorded to the nearest 0.5 of a millimetre. Angular measurements have been made by placing a sheet of glass on the sagittally-sectioned skull and tracing the three points required for each angle on to the glass; the angle is then measured directly on the glass with a protractor. Some of these angles have been

checked from photographs, and the errors of measurement found to be small. Measurements of overall length and breadth and of bizygomatic diameter have been taken on the external surface of the skull after removal of the overlying soft tissues; measurements on the skull-base in the coronal plane have been made on the internal surface of the base after removal of the skull-cap, brain and dura mater; and measurements of diameters and angles in the sagittal plane have been recorded after sagittal section of the cranial base.

Points, dimensions and angles employed

Where no definition of a point or dimension used is given, they are defined as in the adult skull, following the definitions given by Wood Jones (1929).

Basion and opisthion. These are defined as in the adult skull, but the former is a cartilaginous point in the younger foetuses, while the latter is not always easy to define precisely owing to the completion of the posterior border of the foramen magnum by fibrous tissue.

Prosphenion. This is the most anterior point of the presphenoid in the midline on the cranial surface of the skull, but before ossification it has been taken as a point in the midline between the most anterior points on the curved anterior margins of the lesser wings of the sphenoid.

Pituitary point (referred to as pituitary). This is a point in the centre of the floor of the pituitary fossa. The sagittal section of the outline of the fossa is an arc, and the pituitary point is the central point on the arc.

Septal point. This is the most anterior point on the straight lower border of the nasal septum, where it meets the anterior border.

Septum length. The maximum length of the nasal septum.

Septum height. The maximum height of the nasal septum from its lower border to the cribriform plate, perpendicular to the former.

Cribriform plate length and breadth. The maximum length and breadth of the cribriform plate on its cranial surface.

Foramen magnum length. The distance from basion to opisthion.

Foramen magnum breadth. The maximum breadth of the foramen magnum in the coronal plane.

Measurements in relation to the otic capsule

Otic capsule length. The maximum length of the otic capsule along its long axis (which is at about 45° to the sagittal plane).

Interauditory diameter. The minimum distance between the medial margins of the internal auditory meatuses at their openings on the surface of the otic capsule.

Minimum distance between the inner poles of the two otic capsules.

Maximum distance between the outer borders of the two otic capsules on the cranial surface of the skull.

Minimum distance between the posterior margins of the superior semicircular canals.

Maximum distance between the anterior margins of the superior semicircular canals.

Interoptic diameter. The minimum distance between the medial margins of the optic foramina at their internal openings.

Interovale diameter. The minimum distance between the medial margins of the foramina ovalia.

Spheno-ethmoidal angle. The angle basion-prosphenion-nasion.

Foramino-basal angle. The angle prosphenion-basion-opisthion.

Basioccipito-septal angle. The angle basion-pituitary-septal point.

Basioccipito-foraminal angle. The angle pituitary-basion-opisthion.

Note. The latter two angles have been preferred to the spheno-ethmoidal and foramino-basal angles for two reasons:

(1) The prosphenion is difficult to define in the younger foetuses.

(2) The point of angulation between the chordal and prechordal parts of the cranial base appear to be in the pituitary region, so that measurement of this angulation at the pituitary point gives a more direct result than at the prosphenion.

FINDINGS

Between 10 and 40 weeks the overall dimensions of the skull (length, breadth, and auricular height) increase between six- and sevenfold, and there is a change in the form of the head. At 10 weeks the forehead is prominent, while the occipital region is underdeveloped and there is no clear demarcation from the neck. During growth the frontal region becomes less prominent and the occipital region develops, becoming protruberant. The external auditory meatus is thus relatively and absolutely closer to the back of the skull in the younger foetus. The skull is also higher in relation to length and breadth in the young foetus, and becomes squarer with age, due to the development of the frontal and parietal bosses. The lower jaw is more receded in relation to the upper between 12 and 20 weeks than at either the beginning or the end of the foetal period. Other facial changes will be described later.

A. Growth rates within the skull

The dominant structure in the growth of the anterior cranial base is the nasal septum. This doubles its length at 10 weeks by 14 weeks, trebles it by 17 weeks; quadruples it by 22 weeks; it increases fivefold by about 28 weeks, sixfold by 36 weeks, and by birth is between six and seven times its length at 10 weeks. Nasal septum length has been found by the method of least squares to be related to crown-rump length by the allometric formula, $\text{septum length} = 0.23 \times \text{crown-rump length}^{0.86}$.

Most dimensions of the prechordal part of the cranial base have the same growth rate as the nasal septum; they increase six- to sevenfold between 10 and 40 weeks, at the same rate as the septum. This applies to the following dimensions; septum height, length and breadth of cribriform plate, pituitary-prosthion, nasion-prosthion, and otic capsule length. It also applies to the overall length and breadth of the skull and to the cube root of brain weight.

On the other hand, dimensions of the parachordal part of the cranial base have a lower growth rate at all periods; the value at 10 weeks is doubled by 15 weeks, trebled at 22 weeks, quadrupled at 32 weeks, and at birth is only between four and

five times its size at 10 weeks. This applies to pituitary-basion length and to the length and breadth of the foramen magnum. The actual values for the above-mentioned dimensions between 10 and 40 weeks are given in Table 4.

In order to compare graphically the growth rates of the anterior and posterior parts of the cranial base, values for pituitary-nasion have been plotted against those for pituitary-basion for ages between 10 and 40 weeks (Fig. 1).

A linear relationship exists between the two dimensions which can be expressed by the formula $\text{pituitary-basion} = 0.48 \times \text{pituitary-nasion} + 2.5$ (where the values are expressed in millimetres).

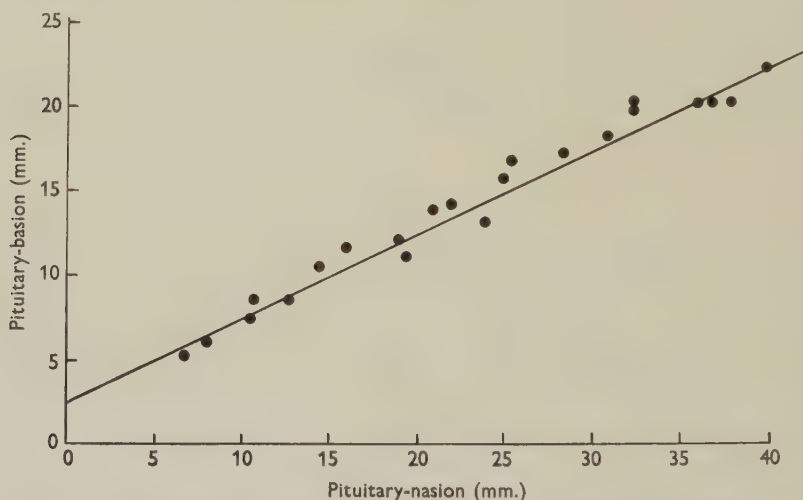


Fig. 1. Mean pituitary-nasion lengths for each group of skulls plotted against mean pituitary-basion lengths for the corresponding groups.

Since the vault of the skull and the brain are growing at the same rate as the anterior part of the cranial base, while the posterior part is growing more slowly, there will be a deficiency in the overall length of the base unless some compensatory structural adaptation exists. This adaptation is in fact a flattening of the cranial base which takes place in the following way:

(1) The angles included between the septal and basioccipital parts of the cranial base, and between the basiocciput and the foramen magnum become progressively less acute.

(2) The occipital squama moves to a more horizontal position relative to the anterior cranial base.

These processes produce the increasing occipital fullness previously noted in the foetal period, which is particularly characteristic of man. As a result of it the posterior cranial fossa, which in the early foetus is funnel-shaped, becomes progressively broader and shallower.

In order to demonstrate this flattening of the cranial base, measurements of the five angles shown in Table 1 have been made in each skull. Although there is considerable individual variation, an analysis of the variance within and between the various age-groups indicates that there is a highly significant regression with

age for each angle ($P < 0.001$). On the assumption that these regressions are linear, the age changes for each angle have been calculated by the method of least squares, and the results are shown in Table 1. Details of the calculations are described elsewhere (Ford, 1955).

Table 1. *Changes in the angles of the cranial base with age*

Angle	Calculated value at 10 weeks (degrees)	Calculated value at 40 weeks (degrees)	Increase between 10 and 40 weeks (degrees)
Spheno-ethmoidal	131.5	150.5	19.0
Basioccipito-septal	104.9	122.4	17.5
Basion-pituitary-nasion	135.4	149.0	13.6
Foramino-basal	120.2	143.0	22.8
Basioccipito-foraminal	109.0	135.0	26.0

The first three angles are all included between the prechordal and chordal parts of the cranial base, but are measured from different fixed points. The degree of change in the first two is very similar, and the discrepancy of the third is probably due to upward movement of the floor of the pituitary fossa during growth, secondary to thickening of the basisphenoid, which may mask some of the change in this angle. The latter two angles record the angulation of the foramen magnum to the basiocciput, and give comparable values. Thus between 10 and 40 weeks the angle between the prechordal and chordal parts of the cranial base opens out by $17-19^\circ$, while that between the basiocciput and the foramen magnum opens out by about 25° , so producing a flattening of the whole cranial base.

It follows from this that dimensions of the foetal skull such as basion-nasion, basion-prosthion, and basion-menton, which are functions of the growth of both the prechordal and the chordal parts of the cranial base, and of the angulation between them, will have a growth rate intermediate between those of the anterior and posterior parts of the cranial base, as reference to Table 4 will show.

B. *Lateral growth in the skull base*

The relative speeds at which the optic and auditory foramina, and the foramina ovalia move laterally, compared with lateral growth of the skull as a whole, is shown in Table 2.

Table 2. *Relative rapidity of lateral growth*

		Overall width	Interoptic diameter	Interovale diameter	Interauditory diameter
Age (weeks) at which 10-week size is	$\times 2$	14	14	15	13
	$\times 3$	17	18	22	16
	$\times 4$	22	34	32	22
	$\times 5$	28	40+	40	30
	$\times 6$	36	—	—	40

The optic foramina move laterally at the same relative speed as the skull as a whole up to 18 weeks, but thereafter their rate of separation from each other is markedly slowed; this is related to the fusion of the orbito-sphenoids with the presphenoid, after which movement of the foramina can only take place by

differential absorption and accretion of bone at their margins, so that they move relatively closer to the midline.

The foramina ovalia move laterally at the same relative speed as the skull as a whole up to about 20 weeks. Their lateral movement is slower in the succeeding 6 weeks, after which they again move laterally at the same relative speed as the skull as a whole.

The auditory foramina also grow laterally at the same speed as the skull as a whole up to 18 weeks when the otic capsules ossify; after this they come to lie progressively closer to the midline, maintaining a constant relationship to the foramen magnum which is expanding more slowly than the skull as a whole, and is therefore becoming smaller in proportion to overall skull size.

C. Growth of the otic capsule

The measurements previously described between the two otic capsules at various points, and the interaural diameter, have been halved to give the distance of each of the points concerned from the mid-sagittal plane. Since the angle which the long axes of the two otic capsules make with each other is about 90° throughout the foetal period, the distances at these points from the midline is proportional to their distance from each other along the long axis of the otic capsule. The results obtained are summarized in diagrammatic form in Fig. 2.

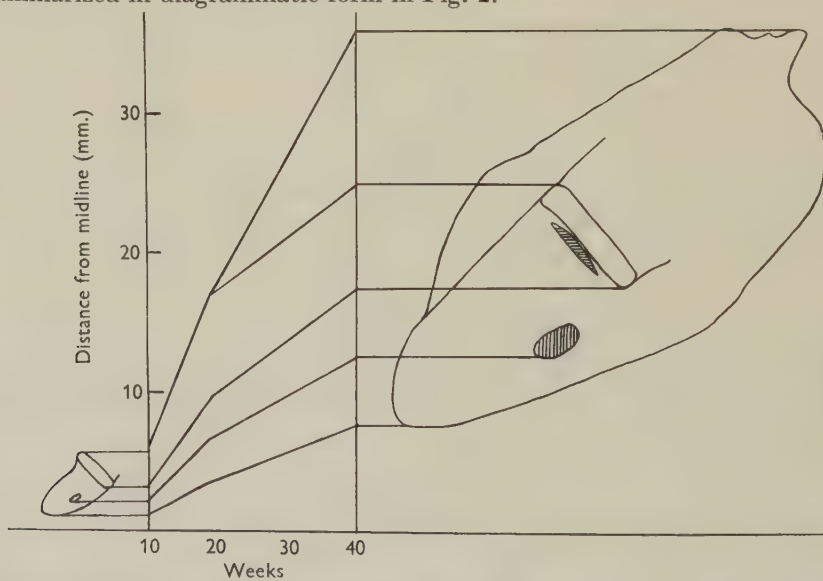


Fig. 2. Diagram of the growth of the otic capsule and petrous temporal bone, showing the distances from the midline of the points described in the text at ages between 10 and 40 weeks. The alteration of the mode of growth which takes place at the time of ossification can be seen.

Up to about 18 weeks the otic capsule grows interstitially in cartilage at the same rate as the enclosed internal ear. At 18 weeks the latter has attained virtually its adult size (Bast & Anson, 1949), the capsule is rapidly ossified throughout, and interstitial growth ceases. The petrous temporal bone has to increase in size to

fulfil its secondary function of providing a floor for the skull; this is done by addition of bone at its lateral and medial ends. The bone added laterally, which is much more than that added medially and which amounts at birth to more than half the total length of the bone, is added from growth cartilage which is present at the postero-lateral margin of the bone and represents the most lateral part of the otic capsule, which remains unossified, plus that part of the tectum posterius which is attached to the postero-lateral margin of the otic capsule. The bone added medially, however, is laid down from the periosteum, the cartilaginous bridge which originally joined otic capsule and basisphenoid having been by this stage replaced by fibrous tissue. As a consequence of this mode of growth the internal ear comes to lie relatively nearer to the median plane during the last 20 weeks of prenatal life.

D. Facial growth

Comparison has been made of upper facial height (nasion-prosthion), facial depth (represented by nasal septum length) and facial width (bimalar diameter). Means of all measurements in the groups 10-15, 16-20, 21-30 and 31-40 weeks have been taken, and Table 3 gives the ratios of the dimensions to each other for each group.

Table 3. *Changes in facial proportions with age*

Age group (weeks)	Depth Height	Width Height	Width Depth
10-15	1.30	1.92	1.58
16-20	1.32	2.26	1.75
21-30	1.15	2.13	1.89
31-40	1.23	2.30	1.93

This indicates that width is increasing in relation to both height and depth, while height is also increasing in relation to depth. The increase in height relative to depth is due to the growth of the alveolus, since facial height is compounded of septum height and alveolar height, and it has been seen that the proportions of the nasal septum are constant during the foetal period. The relative increase is not as marked as might be expected, and this is due to slight movement downward of the nasion relative to the nasal septum. The even greater relative growth in width is related to orbital development.

In order to study prognathism the values for the angle pituitary-nasion-prosthion have been analysed, and it has been found that there is no statistically significant change in the angle with age, the mean value being about 78° , although there is a slight tendency for the value to increase in the last 10 weeks, due to alveolar development. This implies that the degree of true (as opposed to alveolar) prognathism of an individual depends on the position of the alveolus in relation to the nasal septum, and that this is fixed by the beginning of the foetal period.

The dimensions basion-prosthion and basion-menton have been compared, and it has been found that basion-menton length is relatively less in relation to basion-prosthion length between 12 and 20 weeks than before or after this period. This corresponds to the period at which the lower jaw is obviously receded in relation to the upper, and is related to the development of the secondary cartilages of the mandible.

Table 4. *Mean values of certain dimensions of the foetal skull for age-groups between 10 and 40 weeks*

(All diameters expressed in millimetres)

Age group (weeks)	Maximum skull length	Maximum skull width	Brain weight in milligrams	Nasal septum length	Nasal septum height	Cribriform plate length	Cribriform plate width	Pituitary-nasion	Pituitary-prosthion	Nasion-prosthion	Otic capsule length	Basion-nasion	Basion-prosthion	Basion-menton	Pituitary-basion	Foramen magnum length	Foramen magnum breadth	No. of foetuses in group
10	17	11.5	—	5.25	2.75	3	2	6.75	7	4.75	6	10.75	9.75	8.75	5.25	5	4.5	2
11	19	16	11.3	6.5	3	3	2	8	8.5	4.5	6	11.5	10	9	6	5.5	4.5	2
12	27	22	16	8.5	3.5	5	3	10.5	10	6.5	8.5	15	13	11.5	7.5	6	5	1
13	28.25	23	18.7	9.25	4.75	4.5	3.75	10.75	11	7	8.5	17.75	14.75	12	8.5	6.5	5.5	2
14	34	27.5	21	11	5.5	5.5	4.5	13	14	9	10	21	17.5	14	8.5	8.5	6.5	1
15	36.5	29.5	22.2	13	6	6	4	14.5	15.5	10.5	12	24	21	17	10.5	8.5	7.5	3
16	43	35.25	25.3	14.5	6.5	7	4.5	16	17.5	11	13	25.5	22.5	19.5	11.5	9	7.5	3
17	50	40	34.5	16	6.5	7.5	5	19	21	13	16.5	28	25.5	18	12	10	8	1
18	50	39.5	31.5	18	8	8.75	6	19.5	21	13.5	15.5	29.5	24.5	23.75	11	11	9	2
19	52	42	34.5	18.5	8	10.25	6.5	21	23	14.75	16	33.5	29	24.5	13.75	12.75	9	2
20	54	42	—	20	9.5	10.5	6	22	23	14	15	34	29	22	14	15	10	1
21	56	49	38.5	19	9.5	10	6	24	26	17	16	35.5	31	27	13	13.5	11	1
22	64	49	42.5	21	9.5	11	7.5	25	25.25	19	16.75	37	32	28	15.5	14.5	11.5	2
24	75	61	45.5	23.5	10.25	12	7.5	25.5	28.5	20	20.25	41	34.5	31	16.5	15	12	2
26	75.5	62.5	47	24	12	13	8.5	28.5	31.5	21	21.5	44.5	39	34	17	17	12.5	3
28	85.5	67.5	54	26	12	14.25	9	31	33.5	23	24	45	41	37	18	17.5	13.5	2
30	94.5	73	58.5	28.5	13	11	8	32.5	38.5	24	25	47	42.5	40	19.5	20	14	3
32	98	74.5	64	30.5	14	13	8.5	32.5	36.5	25	27	50	46	42.5	20	20	14	7
34	101	76	—	32	16	16	9	36	38.5	27	30	53	47	44	20	21	15	8
36	101.5	79.5	—	34.5	16	15	9.5	37	41	26	34	54	50	47	20	21	17	4
38	108	80	69.5	34	16	15.5	10.5	38	42.5	27.5	35	55.5	53	49.5	20	22	18	13
40	110	87	73.5	35	16	15.5	10.5	40	46	30	36.5	59.5	57	52.5	22	24	21	11

DISCUSSION

The observations on the relative growth rates of the prechordal and chordal parts of the cranial base supplement those of Ortiz & Brodie (1949) who found that in the newborn baby growth in the anterior part of the cranial base is more rapid than in the posterior part. The growth rates appear at first to contradict the law of developmental direction which states that, in general, development (including growth and differentiation) in the long axis of the body appears first in the head region and progresses towards the tail. Since the growth rate of any part or organ is highest when development begins, and declines progressively with age, parts more recently developed will be growing relatively more rapidly at any given stage of foetal development than parts developed earlier. The more rapid growth of the prechordal part of the cranial base implies that it develops later than the chordal part. This does not contradict the law if it is assumed that this is reversed for the prechordal part of the skull as Kingsbury (1924) has suggested, on qualitative studies, that it may be; since the anterior end of the notochord marks the primitive rostral extremity of the head, the prechordal part of the cranial base is both phylogenetically and ontogenetically more recent than the chordal part, and this accounts for its more rapid growth at any given stage of development. The law may thus be amended to state that development commences at the anterior end of the notochord, and progresses both rostrally and caudally from that point.

The changes in the angles of the cranial base in foetal life are brought about mechanically by the expansion of the cranial contents; since the growth of the posterior part of the cranial base is slower than that of the brain, flattening of the cranial base must take place in compensation. If the mechanical force of brain expansion is absent, as in anencephalus, or is reduced, as in microcephalus, the angle between the prechordal and chordal parts of the cranial base often remains a right angle, as in early foetal life (Augier, 1931). In post-natal life the pre-natal change is reversed in man and most primates (Duckworth, 1915), the foramino-basal angle becoming smaller. This again is probably for mechanical reasons, the growth of the cranial base, particularly at the spheno-occipital junction, being more prolonged than that of the brain, and so partially reversing the earlier changes.

It has been seen that the chondrocranium, with those foramina for the cranial nerves which develop in relation to it, expands laterally at the same rate as the skull as a whole up to 18 or 20 weeks, but thereafter comes to lie relatively closer to the midline; this reflects the more rapid growth of the cerebral hemispheres at this time compared with the brain-stem, from which most of the nerves are arising. Owing to this rapid cerebral expansion most of the growth in width of the base of the skull after 20 weeks takes place laterally in the membrane bones.

The dominant feature of facial growth during foetal life is the cartilaginous nasal capsule. The position of the upper alveolus in relation to the nasal septum is already fixed by early foetal life, so that the degree of true (as opposed to alveolar) prognathism does not alter during foetal life. The position of attachment of the alveolus to the nasal septum is characteristic in man, being relatively less rostral than in other primates; this is due to the small size of the premaxilla, and to its early fusion with the maxilla, and it explains the projecting nose and prominent nasal spine of man. In other primates the premaxillae grow forward beyond the nasal septum, which becomes submerged in the face.

The recession of the lower jaw which is conspicuous between 12 and 20 weeks is related to the mode of growth of the mandible. In the early foetal period forward growth of the mandible is due to the growth of Meckel's cartilage; as development proceeds this becomes smaller and less important, and the phylogenetically and ontogenetically more recent temporo-mandibular joint and secondary cartilage of the mandibular condyle are developed, from which most of the future forward growth of the mandible takes place. It is during the period when Meckel's cartilage is becoming relatively small and insignificant, whilst the mandibular condyle has not fully assumed its growth function, that mandibular growth lags behind that of the upper jaw. If there is interference with growth at this stage the temporary inequality in the positions of the two jaws may become fixed.

SUMMARY

1. The growth of the foetal skull between 10 and 40 weeks has been studied by measurements on a series of dissected formalin-preserved foetal heads.

2. Overall skull size, brain size and the anterior (prechordal) part of the cranial base increase between six- and sevenfold in linear dimension between 10 and 40 weeks, while dimensions of the posterior (chordal) part of the base only increase

between four- and fivefold. To compensate for this slower growth of the posterior part of the cranial base, the angles between the pre- and para-chordal parts of the base, and between the basiocciput and the foramen magnum, become flattened, resulting in increased prominence of the occiput.

3. The parts of the cranial base derived from the chondrocranium come to lie relatively closer to the midline after 18–20 weeks. Growth of the otic capsule is described; this, after ossification, grows mainly at its postero-lateral end from growth cartilage, but there is some subperiosteal accretion of bone at the antero-medial end.

4. Facial width increases relative to both height and depth, while height also increases relative to depth, but the degree of prognathism remains constant. Growth of the lower jaw lags behind that of the upper between 12 and 20 weeks while the condylar growth centre is not yet fully developed.

This work was made possible by a grant from the St Thomas's Hospital Endowment Fund.

I am grateful to Professor D. V. Davies for providing facilities and material for the work, and also to Professors R. E. M. Bowden, J. D. Boyd, R. J. Harrison and R. D. Lockhart for foetal material.

In the statistical analyses I was advised by Mr M. J. R. Healey of the Rothamsted Research Station, who also did most of the computations, for which I am very grateful.

I also wish to thank Mr G. A. Wooding for carrying out the photographic work.

REFERENCES

- AUGIER, A. (1931). In Poirier & Charpy, *Traité d'Anatomie Humaine*, 4th ed., Tome 1, Fasc. 1, p. 627. Paris: Masson et Cie.
- BAST, T. H. & ANSON, B. J. (1949). *The Temporal Bone and the Ear*, p. 245. Illinois: Charles C. Thomas.
- DUCKWORTH, W. H. L. (1915). *Morphology and Anthropology*, 2nd ed. vol. 1, p. 232. Cambridge University Press.
- FORD, E. H. R. (1955). The growth of the foetal skull. M.D. thesis, University of Cambridge.
- KINGSBURY, B. F. (1924). The significance of the so-called law of cephalocaudal differential growth. *Anat. Rec.* **27**, 305–321.
- ORTIZ, M. H. & BRODIE, A. G. (1949). On the growth of the human head from birth to the third month of life. *Anat. Rec.* **103**, 311–333.
- SCAMMON, R. E. & CALKINS, L. A. (1929). *The Development and Growth of the External Dimensions of the Human Body in the Fetal Period*. Minneapolis, University of Minnesota Press.
- STREETER, G. L. (1920). Weight, size, and age of human embryos. *Contr. Embryol. Carneg. Instn.* **11**, 143–170.
- WOOD JONES, F. (1929). Measurements and landmarks in physical anthropology. *Bull. Bishop Mus., Honolulu*, no. 63. Hawaii.

THE STRUCTURE AND DEVELOPMENT OF CRANIAL AND FACIAL SUTURES

By J. J. PRITCHARD, J. H. SCOTT AND F. G. GIRGIS

Anatomy Department, Queen's University, Belfast

It is surprising, in view of the controversy which has long raged about the functional role of the sutures in the growth of the skull, that so little attention has been paid to their histological structure and development. The most comprehensive study is that of Sitsen (1933), who describes the development of the lambdoid suture in man between the 8th month of foetal life and 12 years. Mair (1926), Petersen (1930), Troitsky (1932), Bernstein (1933), Weinman & Sicher (1947), Moss (1954), and Scott (1954) have made more limited contributions to the literature.

As it was evident that much more detailed and accurate information was required in order to provide the necessary basis for experimental work on the morphogenesis and functions of the sutures, it was decided to investigate the development and structure of a variety of sutures in available foetal, young and adult material.

MATERIALS AND METHODS

Serial sections through the heads, or parts of the heads, of several stages in the development of six species were used. These comprised: nine human specimens, ranging from 45 mm. C.R. to full term; seventeen sheep from 48 mm. C.R. to 18 months; six pigs from 30 mm. C.R. to 5 months post-natal; five cats from 40 mm. C.R. to 57 days post-natal; four rabbits from 35 mm. C.R. to full term and an adult; and a series of ninety-three rats ranging in age from the 15th day of foetal life to 1 year.

More than one half of the total number of specimens were cut coronally, the remainder were chiefly cut in the sagittal plane but a few were transverse. Most of the series were stained by Masson's or Mallory's methods, or with Weigert's haematoxylin and van Gieson. Some sections were impregnated with silver by Wilder's method, and a few were stained with Harris's haematoxylin and eosin or safranin. Some alcohol-fixed rat heads were also cut and stained for alkaline phosphatase by Gomori's method and for glycogen by the periodic-acid-Schiff method.

The Masson and Mallory stained sections were most useful for determining the patterns of relatively coarse collagenous fibres. Wilder's silver method gave more precise information about the finest collagen fibres. Weigert's haematoxylin and van Gieson was especially useful in the correlation of cellular with fibrous patterns. Sites where active osteogenesis was in progress were clearly demonstrated in sections stained for alkaline phosphatase and glycogen.

METHODS

All the sutures examined went through essentially similar stages in their development. It will be more convenient, therefore, and less repetitive, to describe the observations stage by stage, rather than to attempt to give the chronological history of each suture separately.

(1) *Stage of approaching bone territories.* (i) It is to be noted that the term 'bone territory' includes the definitive bone, the preosseous cambial layer and the fibrous periosteum. (ii) A 'suture' is to be regarded as the entire complex of cellular and fibrous tissues intervening between, and surrounding, the definitive bone edges.

(2) *Stage of meeting of the bone territories.*

(3) *Early growing stage.*

(4) *Late growing stage.*

(5) *Adult stage.*

(1) *Stage of approaching bone territories*

Two main types may be distinguished, viz. (a) that in which the bones approach one another through loose mesenchymatous tissue, as in the face (Pl. 1, fig. 1); and (b) that in which the bones approach within a preformed fibrous membrane, as in the cranial vault (Pl. 1, figs. 2, 4).

(a) *The facial skeleton*

Each approaching 'bone territory' shows three well-marked zones: (1) an outer periosteal fibrous capsule, (2) a periosteal cambial layer, (3) an inner plate of woven membrane bone (Pl. 1, figs. 1, 9).

The *periosteal fibrous capsule* shows an essentially tangential arrangement of collagenous fibres interspersed with similarly orientated elongated fibroblasts (Pl. 1, fig. 9). At the advancing edge of the bone territory these fibres run at right angles to the plane of the bone: elsewhere they are parallel to it.

The *cambial layer* shows fine collagen bundles running in a radial direction from the bone to the fibrous capsule. These are the osteogenetic fibres, some of which will later develop into stout Sharpey's perforating fibres. The cambial layer is very cellular, showing an outer zone of small rounded proliferating elements (pro-osteoblasts) and an inner zone next to the bone of larger cells, pyriform or polygonal in shape (definitive osteoblasts) (Pl. 1, fig. 9).

In a previous paper (Pritchard, 1952) the cytology of these cells has been considered in detail. Suffice it to state here that the pro-osteoblasts show numerous mitotic figures, and are rich in glycogen (Pl. 2, fig. 16), and alkaline phosphatase, while the definitive osteoblasts do not show mitotic figures, contain no glycogen, somewhat less phosphatase than the pro-osteoblasts, intense cytoplasmic basophilia, a very large Golgi apparatus and numerous elongated mitochondria.

The cambial layer is evidently the site of very active osteogenesis, the new bone being of the woven variety. It is evident also that the fibrous periosteal capsule must be expanding to keep pace with the growing bone.

In the loose cellular mesenchyme between the bone territories the future location of the definitive suture is not indicated by any special differentiation of cells or fibres

(Pl. 1, fig. 1), although in the palate a very thin strand of condensed mesenchyme runs between the approaching bone territories. This may possibly serve to guide the bones towards each other (Pl. 1, fig. 1).

(b) The cranial vault

Unlike the bones of the facial skeleton, those of the cranial vault approach each other within an already differentiated fibrous membrane, the ectomeninx, desmocranium, or fibrous brain capsule (Pl. 1, figs. 2, 4). Each bone is surrounded by a cambial layer of osteogenetic cells and fibres similar to that described for the facial skeleton, outside which the fibrous periosteal layers, represented by the pericranium and dura mater, are well differentiated. These two membranes, however, do not encapsulate the cambium at the extreme edge of the bone with fibres running at right angles to the plane of the bone, as in the face. Instead, they join in front of the advancing cambium and run across the region of the presumptive suture parallel to the plane of the bones. The approaching bones with their cambial layers therefore appear to be delaminating the ectomeninx into pericranial and dural strata.

The presumptive suture is thus chiefly composed of parallel fibres continuous with the pericranium and dura mater of the approaching bone territories. The eventual meeting place of the bones is as yet unmarked by any special arrangement of cells or fibres, although in the case of the presumptive sagittal suture the position of the definitive suture can be inferred from the position of the sagittal venous sinus (Pl. 1, fig. 2).

(2) Stage of meeting of the bone territories

Because of the differences between the face and cranial vault in respect of the composition of the bone territories and the structure of the tissue lying between them, sutural junction is effected in two different ways. In the face the fibrous capsules of the bone territories become united by means of two fibrous laminae, an external and an internal, which make their appearance as the territories come close together (Pl. 2, figs. 3, 5). These will be referred to as the *uniting layers*. The rounded extremities of the fibrous capsules, however, retain their separate identities, for a remnant of the loose cellular mesenchymal tissue, which previously lay between the approaching bone territories, intervenes.

The suture at this stage therefore presents *five distinct layers* as one passes from the edge of one bone to the edge of the other, namely the first cambial layer, the first fibrous capsule, the loose cellular middle zone, the second fibrous capsule and the second cambial layer. These will be referred to as the *intervening layers* of a suture as opposed to the aforementioned uniting layers.

The cambial and capsular layers on each side retain the general appearance they had before the bone territories met. They are, therefore, identical in structure, and directly continuous with, the cambial and fibrous layers of the periosteum on the non-sutural surfaces of the bone (Pl. 1, figs. 3, 5).

The meeting of the cranial vault bones is different in that the approaching bone territories are not separated by loose mesenchyme, but are united by the fibrous ectomeninx which is continuous with the periosteal layers of each bone (Pl. 1, figs. 2, 4). Moreover, there are no preformed fibrous capsules as in the facial

skeleton. These, however, rapidly appear on each side as the edges of the bones approach closely.

The width of undelaminated ectomeninx between the bones progressively decreases until the cambial layers on either side are almost in contact, when the remnant begins to split into relatively dense outer and inner uniting layers leaving a looser layer between them (Pl. 1, fig. 4). The way might now seem clear for the cambial layers to fuse across this loose region, but the rapid appearance of a pair of encapsulating fibrous strata prevents this (Pl. 1, fig. 8). These capsules consist of relatively coarse parallel fibres running at right angles to the uniting layers, covering the cambial layers on each side and separated from each other by the loose middle tissue (Pl. 2, fig. 14). The origin of the capsular fibres is difficult to determine with certainty, but the appearances suggest that the more peripheral cambial cells become transformed into fibroblasts around which collagen fibres are laid down.

In this way the sutures of the cranial vault come to possess five intervening and two uniting layers as in the facial skeleton (Pl. 1, fig. 8; Pl. 2, fig. 14).

(3) *Early growing stage*

For some time after the meeting of the bony territories and formation of the suture, all the layers mentioned continue to be well marked and easily differentiated (Pl. 1, figs. 7, 10; Pl. 2, fig. 11). The edges of the bones pass from a unilaminar to a bilaminar or a multilaminar state (Pl. 2, fig. 11) as they increase in thickness by surface accretion on both external and internal surfaces. Diploic spaces are formed, partly by enclosure of the spaces between the laminae of bone, partly by osteoclastic resorption. The cambial layer shows clearly defined pro-osteoblastic and osteoblastic zones; and cellular proliferation, indicated by the frequency of mitotic figures in the pro-osteoblastic zone, is evidently very active. In the cambial zone, also, bundles of osteogenetic fibres emerge from the bone and run radially between the osteogenetic cells to join the tangentially running fibres of the capsular zone. The cambial zone shows intense alkaline phosphatase activity and conspicuous glycogen storage, similar in distribution to that previously described, but the fibrous capsular zone and the loose middle zone are practically devoid of both these substances (Pl. 3, figs. 18, 19). The suture at this stage is evidently the site of rapid marginal extension of the adjoining bones, two growth centres being apparent, viz. the pair of cambial zones surrounding the edges of the bones. The fibrous capsular zones and the middle zone, however, show little evidence of active cellular proliferation, although the capsular tissue must in fact be growing in order to accommodate the ever increasing volume of the contained osseous tissue.

(4) *Late growing stage*

The bone edges are now thicker and more compact (Pl. 1, fig. 6; Pl. 2, fig. 12) and have a smoother surface. Instead of pointed trabeculae of woven bone projecting radially towards the suture, as in the early growing period, compact lamellar bone is now being added tangentially. Much of the earlier formed woven bone has been removed and replaced with lamellar bone containing simple Haversian systems. The cement lines near the suture edge, moreover, are mostly evenly curved and

parallel, indicating a more or less continuous process of surface accretion. Reversal lines, and Howship's lacunae with osteoclasts, are occasionally seen, indicating local areas of past and present resorption, probably associated with internal remodelling. There is little evidence that extensive surface resorption plays an important role in suturogenesis, even in squamous over-lapping sutures. The sutural connective tissue still shows the five intervening zones already described, but their proportions have altered. The cambial layer is reduced to a single layer of osteoblasts, and in places these are represented only by inconspicuous cells flattened against inactive bone surfaces. The two fibrous capsular layers are denser, but the direction of the fibres remains tangential to the sutural faces of the bones (Pl. 2, fig. 12). Some strong bundles of radially directed fibres emerge from the bone surfaces and end in the capsular layers (Pl. 1, fig. 6). These should now be interpreted as Sharpey's fibres, binding the bone to the capsule, and not as osteogenetic fibres, the term more appropriate in the early growing stage. Very similar, but weaker, Sharpey's fibres, are found in the remnant of the cambial layer of the non-sutural periosteum.

The fibrous periosteum covering the non-sutural surfaces of the bones now has the appearance of splitting into two layers as the suture is reached, the outer of which runs straight across the external boundary of the suture from one bone to the other to form one of the uniting layers of the suture; while the inner turns into the suture to form one of the fibrous capsules, which in turn joins the inner layer of the periosteum on the other side.

The middle zone is now very vascular, exhibiting large thin-walled sinusoidal vessels which join the diploic vessels, the dural sinuses and the pericranial veins. Its fibres are in the form of independent bundles which course between the blood vessels from one fibrous capsule to the other. Their predominant direction is at right angles to the capsular fibres, but some are oblique. On the whole the middle zone is much less densely fibrous than the capsular zones on either side of it. In parts of some sutures, however, where the vessels are scanty, the middle zone is inconspicuous, for the two capsular zones are very close together and partly fused.

At this stage growth of the bones at the suture is evidently very slow and chiefly directed towards rounding and smoothing the bone edges.

(5) *Adult stage*

The bone edges now show no signs of growth activity, and the cambial layer is reduced to a single layer of inconspicuous flattened cells (Pl. 2, figs. 13, 17). The potential osteogenetic nature of these cells, nevertheless, is proved by their rapid re-awakening to activity in the presence of a fracture in a distant part of the bone. The fibrous capsules, on the other hand, are still distinct (Pl. 2, fig. 13), and are separated from one another by a middle zone which is even more vascular than before (Pl. 3, fig. 22).

The general direction of the fibres is the same as in the growing stages, but now numbers of Sharpey's fibres can be traced in continuity from one bone to the other (Pl. 2, fig. 15). Despite these the strongest bond of union is through the uniting layers at the external and internal boundaries of the suture.

CARTILAGE

In several sutures cartilage has been found at the margins of the bones or in the sutural tissues proper. It was most common in the sagittal and mid-palatal sutures at the end of the period of rapid growth, and was of two types.

The first type occurred as irregular islands or areas of large-celled cartilage with scanty matrix, interspersed with, or capping the trabeculae of woven bone at or near the sutural edges. In one specimen such cartilage ran from one parietal to the other across the sagittal suture (Pl. 3, fig. 21).

The second type presented a more orderly appearance. In the palate of the rat, for example, both cambial layers of the suture were temporarily transformed into expanded epiphysis-like masses covering the margins of the bones. Each mass showed a regular gradation from pro-cartilage near the middle of the suture, through definitive hyaline cartilage, to hypertrophic cartilage adjacent to the bone (Pl. 3, fig. 20). The two cartilage masses were separated in the middle of the suture by a narrow band of compressed fibrous tissue representing the fused capsular and middle zones.

In the rat a sagittal rod of cartilage was found transiently just above the sagittal venous sinus (Pl. 1, fig. 2), but it disappeared soon after birth, and as it lay beneath that part of the ectomeninx in which the definitive suture was destined to form, it evidently plays no part in suture formation. This cartilage appeared to be a forward extension from the tectal region of the chondro-cranium.

SYNSTOSIS

In several specimens localized areas of synostosis across the suture were found. This was not uncommon in the sagittal suture of the rat at any time after the third week from birth. Other examples were found in the mid-palatal suture. In one such specimen the connecting bone contained hypertrophic cartilage cells (Pl. 2, fig. 22), suggesting that bony union had been preceded by cartilaginous union as in the example previously mentioned. In the sagittal suture *partial* synostosis was sometimes found (Pl. 3, fig. 24), the bones being united on the dural but not on the pericranial side.

GROSS SUTURAL MORPHOLOGY

This paper is not concerned with the detailed description of adult sutural patterns. It is to be noted, however, that a similar five-layered arrangement of cells and fibres was found in simple harmonic sutures, in complicated denticulate sutures (Pl. 1, fig. 10) as well as in squamous overlapping sutures, and the general observations reported apply equally well to all these varieties.

CHRONOLOGY

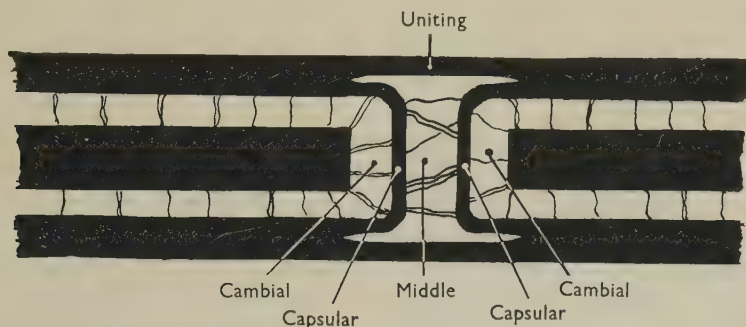
The absolute time-course of suture differentiation varied considerably in different sutures, in different animals and even in different parts of the same suture, but all went through essentially similar stages in their development. This study was not competent to determine absolute, or even relative, growth rates at different sutural

margins, nor was interest especially directed to the widely different times at which suture margins cease growing. It was apparent, however, that the mode of growth, if not its rate, conformed to a common pattern in all cases.

CONCLUSIONS AND DISCUSSION

Fundamental structure of cranial and facial sutures

In the animals studied, the sutures throughout their development exhibited *five* distinct layers of cells and fibres *between* the edges of the adjoining bones. In addition, the outer and inner boundaries of the suture were marked by fibrous strata which ran without interruption from the fibrous periosteum of one bone to that of the other. Thus we have *five* intervening layers and two *uniting* layers comprising the basic structure of a suture (Text-fig. 1).



Text-fig. 1. Diagram to show the general construction of a suture.

All these layers have, at one time or another, been described by previous workers, but no single worker seems to have recognized them all. Thus Sitsen (1933), in the human lambdoid suture, describes three intervening layers in the newborn, which seem to correspond to the cambial layers and the middle layer of our description, but he fails to recognize the capsular layers. At a later stage he describes only one layer, apparently the combined capsular and middle layers. Troitsky (1932) describes the uniting as well as the cambial layers. He also mentions a 'non-osteogenic' middle layer, but he does not differentiate this into capsular and middle zones. Bernstein (1933) mentions a loose middle layer and the perforating fibres of the cambial layer. Weinman & Sicher (1947) mention three layers, the cambial layers not being described. Moss (1954) describes a suture as possessing three layers at an early stage of development, corresponding to our capsular and middle layers, but in the adult he recognizes only a single (fibrous) layer. Scott (1954) describes four layers, the cambial and the capsular on each side, but does not mention the middle layer.

It is probable that the authors mentioned missed some of the layers because selective fibre stains were not always employed. Both Scott and Troitsky stress the continuity of the cambial and capsular layers with the corresponding cambial and fibrous layers of the periosteum. This important fact for the understanding of bone growth at sutures seems to have been overlooked by other investigators.

Initial development of sutures

This investigation has shown two differing modes of suture formation in the foetus. In the formation of the facial sutures the cambial and capsular layers are present around the advancing edges of the bones well before they meet, but the uniting layers are not differentiated until the sutural junction is about to be effected. In the skull vault, on the other hand, the edges of the bones are provided with a cambial layer, but no capsular layer, and the uniting layers are already present (as the undivided ectomeninx), although not yet delaminated from each other. In the skull vault it is the capsules which appear as the bones meet. The middle layer in each case arises from the mesenchyme or ectomeninx which lies between the bone territories.

Each bone territory thus contributes cambial and capsular layers to the suture. The middle layer and the uniting layers, on the other hand, are provided by the inter-territorial tissues. Of previous workers only Scott (1954) seems to have appreciated that a suture is constructed from the periosteal layers of a pair of osseous territories, though he failed to recognize the contribution from the tissue between the territories.

At this point it is of interest to compare and contrast suture development with that of a diarthrodial joint. The three-layered *interzone* of diarthrodial joints described by Haines (1947) is paralleled by our five-layered *intervening zone*; his chondrogenous layer corresponding to our combined cambial and capsular layers, while his intermediate loose layer and our middle layer are evidently homologous. But Haines describes the disappearance of the chondrogenous layer as it is transformed into articular cartilage, while the corresponding layers in sutures persist. Haines's loose intermediate layer breaks down to form part of the synovial cavity but the middle layer in a suture persists and becomes very vascular, besides containing fibres which run from one bone to the other.

The fibrous capsule of a diarthrodial joint appears homologous with the uniting layers of a suture, but, just as Haines distinguishes two kinds of capsular development, viz. that arising from non-blastemal mesenchyme (the usual method) from that arising from the skeletal blastema (in fish, mammalian hyoid), so in our study we find the uniting layers of facial sutures originate from non-blastemal mesenchyme but in the cranial vault from the blastemal ectomeninx.

No special differentiation or orientation of cells or fibres marks the future site of meeting of the bone territories before the sutures have been established. This suggests that the position of the sutures is determined by, and is not determined until, the meeting of the bones, a conclusion which is supported by experimental work on the rat in which the skull has been damaged *in utero* leading to abnormal sutural patterns after birth (Girgis & Pritchard, 1955). Troitsky's (1932) finding of normal sutural patterns following damage to the neonatal skull is attributable to the sutures having been already established at the time of experimental interference.

Changes in suture structure during development

As development proceeds profound changes take place in the structure of a suture. The cambial layers, which originally were thick and exhibited several strata of proliferating and differentiating osteogenetic cells, become progressively thinner

until eventually only a single layer of cells, flattened against the surface of the bone, remains. The finer bundles of osteogenetic fibres which ran between the osteoblasts of the earlier active cambium are gradually replaced by stout Sharpey's fibres, some of which pass directly from one bone to the other. The capsular layers become increasingly dense until they form the main bulk of the suture. The middle zone, on the other hand, becomes increasingly vascular.

Nevertheless, in spite of these changing appearances, the original five-layered structure of the suture is always discernible. Sitsen (1933) and Moss (1954), who have described a simplification of the sutural structure down to a single 'sutural membrane' in the adult, have failed to appreciate the remnant of the cambial layers, and, unaccountably, have not apparently noticed the very vascular middle layer.

The changes in sutural histology just described are paralleled by changes in the appearance of the sutural faces of the bones. At an early stage pointed trabeculae of woven bone project into the thick cellular cambial layers. The trabeculae are covered with large active osteoblasts and the classical appearance of rapid bone formation is found. Later the bone margin presents a smoother, rounded face to the suture and a single epithelioid layer of active osteoblasts covers its surface. The superficial layers of the bone consist now of circumferential lamellae containing here and there simple Haversian systems. In places osteoclasts in Howship's lacunae witness to the remodelling which is taking place, whereby the earlier woven bone is removed and replaced by lamellar bone. Later still the bone edge is quite smooth and the osteogenetic layer is inactive. These changes in the structure of the bone (but not of the cambium) are described in detail by Bernstein (1933) and Sitsen (1933).

The mode of origin of the interlocking of the bone margins in complex sutures has not been specially investigated here, so that a firm opinion cannot be expressed as to whether the denticulations arise by differential accretion only or by combined accretion and resorption. The surprising infrequency of signs of osteoclastic erosion at sutural margins of the bones, however, would lead us to suspect that differential accretion is the more probable, a conclusion also reached by Mair (1926), Bernstein (1933), Massler & Schour (1951), and Baer (1954).

Functional significance of sutural morphology

The histology of the suture suggests that it has two main functions, viz. that it is a site of active bone growth, and that it is at the same time a firm bond of union between the neighbouring bones, which nevertheless allows a little movement.

That sutures have this dual function has been expressly stated by Bernstein (1933), Giblin & Alley (1944), Massler & Schour (1951), Moss (1954), Baer (1954), and Scott (1954).

That active bone formation takes place at sutures is rendered very probable from the fact that the cambial layers show the classical histological picture of new bone formation in an even more marked degree than the cambial layers of the periosteum on the non-sutural surfaces of the bones. The intense phosphatase activity and glycogen storage of the sutural cambial layers further reinforces this view. Moreover, direct measurement of the rate and amount of bone growth at sutures is possible by the employment of markers. Thus, von Gudden (1874) showed that holes

drilled in the parietal and frontal bones of young rabbits moved apart in subsequent growth. Troitsky (1932), using silver wires placed in the skull vault of puppies, Giblin & Alley (1942), using trephine holes filled with lamp-black, and Massler & Schour (1951) and Baer (1954), employing alizarin staining, have confirmed this separating growth at sutures. Baer made an extensive series of measurements which demonstrated the over-riding importance of bone formation at sutures in the enlargement of the skull; and also the vital role of differential growth at different sutural margins in determining the changing form of the skull during ontogeny. The failure of von Mijsberg (1932) and Brash (1934) to demonstrate such growth is probably accounted for, as Moss (1954) argues, by their using animals of an age at which cranial expansion had virtually ceased.

Despite this majority view that bones grow at their sutural margins, the sutural element responsible for growth is in doubt. Bones cannot grow interstitially, and it is generally recognized that soft-tissue growth must take place at sutures to provide the substratum for bone formation, but while Weidenreich (1930), Bernstein (1933), Giblin & Alley (1944), and Weinman & Sicher (1947) think that the middle layer of loose cellular connective tissue is the important proliferating tissue, Troitsky (1932), Massler & Schour (1951), Moss (1954), and Scott (1954), correctly, we believe, attribute this role to the cambial layers. These certainly have the histological appearance of rapidly proliferating tissues, including numerous mitotic figures, but the middle zone has not. The latter has, in fact, the character of a neutral zone between two growth zones. Troitsky (1932) has also stressed the non-osteogenetic nature of the central part of the suture as a means of *limiting* bone growth and thus preventing synostosis. Perhaps of more importance is the fact that the provision of two sutural growth zones, separated by indifferent tissue, should enable growth at each bony margin to be independent of the other as regards rate, direction and duration, so allowing for changes in the shape and proportions of the growing skull (Moss, 1954).

The factors controlling osteogenesis at sutures have often been the subject of speculation. The most generally favoured view is that the expanding brain and continuing intracranial pressure lead to tension in the sutural soft tissues, and bone growth is a direct response to such tension (Weinnold, 1922; Loeschke & Winnold, 1922; Massler & Schour, 1951; Moss, 1954). The experimentally-determined fact that opposing bony margins may have very different growth rates (Giblin & Alley, 1942; Moss, 1954; Baer, 1954), however, suggests that individual differences in inherent growth potential are also involved. Giblin & Alley (1944) and Moss (1954) stress the plasticity of suture growth whereby activity at one suture can compensate for inactivity at another, without the form of the skull as a whole being affected. Scott (1954), besides brain expansion, invokes the cartilages of the cranial base, nasal septum and lower jaw (Meckel's cartilage and the condyloid secondary cartilage) as 'pacemakers' governing sutural bone growth from a distance. Muscle activity associated with mastication may also profoundly affect the shape of the skull (Washburn, 1947), and thus, by inference, sutural activity.

Considered as articulations, the sutures possess the means for resisting gross separation of the bones, while at the same time permitting slight relative movement. The uniting layers are the strongest bond of union between the bones, and are

evidently homologous with the fibrous capsule of diarthroses. The central zone, however, with its weak fibre bundles running in all directions and its sinusoidal blood vessels, could well allow some slight movement of one bone against the other, and so could be regarded as analogous to a synovial joint cavity. In most diarthroses the growth zones of the articulating bones are separated from the joint surfaces, and hence protected from undue mechanical stress (in particular, shearing stress), by the cartilaginous epiphyses. In sutures a similar protective role may well be a function of the fibrous capsules surrounding the cambial zones, cartilage being generally unnecessary because the major stresses are likely to be tensile ones.

The middle zone, in addition to separating the growing regions, and permitting momentary adjustments of one bone relative to the other, probably also enables slow progressive angulation to take place between the bones as the skull alters in shape during growth, thus saving the need for very extensive remodelling (cf. von Mijsberg, 1932). The great vascularity of the middle zone is more difficult to explain. It might simply be a means of filling the unwanted spaces between the fibre bundles without embarrassing movement; it might be a part of the emissary system of veins; on the other hand, it might serve as a hydrostatic cushion between the ends of the bone reinforcing the other protective measures against undue mechanical stresses.

Cartilage in sutures

Cartilage was found for a limited period in some post-natal sutures, especially the sagittal and mid-palatal. The appearance of secondary cartilage in the course of membrane bone development has often been described (de Beer, 1937; Symons, 1952; Dixon, 1953). It is evidently an alternative to bone formation among proliferating osteogenetic cells, and as such it is prominent in fracture repair of long bones (Pritchard & Ruzicka, 1950), and even in parietal fractures cartilage is occasionally formed (Pritchard, 1946). Similar tissue appears in cultures of bone *in vitro* (Fell, 1933). In membrane bones it may function as a growth cartilage (Symons, 1952) for long periods (e.g. mandibular condyle, ends of clavicle) or it may rapidly disappear, either by resorption with or without endochondral replacement, or by direct conversion to bone (Pritchard & Ruzicka, 1950).

The significance of sutural cartilage is debated. Sitsen (1933), who found it in infants under 6 months of age in the lambdoid suture, regards it as the result of particularly strong pressure and shearing stresses between the bones associated with recumbency at this stage of life, for it is well known that fibrous tissues may become cartilaginous when subject to such stresses. The effect of masticatory forces ought perhaps also to be considered in relation to the development of sutural cartilage.

A protective function for such cartilage is also a possibility, for, as is well known, growing bone is intolerant of pressure and shearing stresses. That cartilage is not present at all stages in suture development accords with the view that normally sutural tissues are under tension.

There remains the possibility that cartilage formation is the result of temporary ischaemic conditions associated with rapid growth (cf. Ham, 1930). In this connexion it is of interest that much greater amounts of cartilage appear in fractures of the skull vault when the blood supply to the area is deliberately reduced than under normal conditions of repair (Girgis, 1955, unpublished observation).

Closure of sutures

Obliteration of sutures and synostosis of the adjoining bones, if it happens at all, occurs usually after all growth has ceased. Different sutures and different animals vary greatly in this respect. In the great apes synostosis of all sutures occurs immediately growth has ceased, but in man and most laboratory animals sutures may never completely close (Bolk, 1915). These differences have been attributed to the differences in the degree of development of the masticatory apparatus. In this investigation local areas of synostosis were found in the adult rat sagittal and palatal sutures, and even as early as 3 weeks after birth partial bony union had occurred at some sites in these sutures. Such synostoses are probably without special significance as, in any case, growth at these sutures ceases very early (10–20 days, Massler & Schour, 1951), while compensatory overgrowth at other sutures, as already mentioned, readily occurs.

SUMMARY

1. The structure and development of cranial and facial sutures has been studied histologically in the rat, sheep, pig, cat, rabbit and man.

2. At all stages from their first development up to and including the adult, sutures show *five intervening* layers of cells and fibres between the adjoining bones as well as *two uniting* layers bounding the suture externally and internally.

3. The intervening layers comprise pairs of cambial and capsular layers continuous with the cambial and deeper fibrous strata respectively of the periosteum covering the non-sutural surfaces of the bones, and a middle looser zone. The uniting layers run directly between the outer fibrous layers of the periosteum on each side.

4. Sutures arise somewhat differently in the face and cranium. In the face the cambial and capsular layers are already present before the suture is formed, while the middle and uniting layers are derived from the mesenchyme between the approaching bone territories. In the cranium the capsular layers are not formed until the cambial layers have almost met, while the uniting and middle zones are derived from the delamination of the fibrous ectomeninx between the bones.

5. As a suture matures its cambial layers are gradually reduced to a single layer of flattened osteoblasts, the capsular layers thicken but their predominant fibre direction continues to be parallel to the sutural faces of the bones, while the middle layer becomes increasingly vascular. The uniting layers form the strongest bond of union between the bones.

6. Cartilage, either of the hypertrophic or the hyaline variety, is found at the margins of the bones in some sutures during the neonatal period. Cartilage union across the suture is occasionally found.

7. Areas of partial synostosis were found in some palatal and sagittal sutures both during, and after the completion of, skull growth.

8. It may be deduced from their mode of development and their histological organization, that sutures form a strong bond of union between adjacent bones while permitting slight movements, and at the same time allowance is made for marginal expansion of the bones during the growing period.

We wish to thank the Northern Ireland Hospitals Authority for a research grant to cover the cost of animals and photographic materials used in this work.

REFERENCES

- BAER, M. J. (1954). Patterns of growth of the skull as revealed by vital staining. *Hum. Biol.* **26**, 80-126.
- DE BEER, G. R. (1937). *The Development of the Vertebrate Skull*. London: Oxford University Press.
- BERNSTEIN, S. A. (1933). Über den normalen histologischen Aufbau des Schädeldaches. *Z. ges. Anat., Z. Anat. Entw-Gesch.* **101**, 652-678.
- BOLK, L. (1915). On the premature obliteration of sutures in the human skull. *Amer. J. Anat.* **17**, 495-523.
- BRASH, J. C. (1934). Some problems in the growth and developmental mechanisms of bone. *Edin. Med. J. (N.S.) (IV)* **41**, 305-387.
- DIXON, A. D. (1953). The early development of the maxilla. *Dental Practitioner*, **3**, 331-336.
- FELL, H. B. (1933). Chondrogenesis in cultures of endosteum. *Proc. Roy. Soc. B*, **112**, 417-427.
- GIBLIN, N. & ALLEY, A. (1942). A method of determining bone growth in the skull. *Anat. Rec.* **83**, 381-387.
- GIBLIN, N. & ALLEY, A. (1944). Studies in skull growth: coronal suture fixation. *Anat. Rec.* **88**, 143-153.
- GIRGIS, F. G. & PRITCHARD, J. J. (1955). Morphological status of cranial sutures. *J. Anat., Lond.*, **89**, 577.
- VON GUDDEN, B. (1874). *Experimental-Untersuchungen über das Schädelwachstum*. Munich.
- HAINES, R. W. (1947). The development of joints. *J. Anat., Lond.*, **81**, 33-55.
- HAM, A. W. (1930). A histological study of the early phases of bone repair. *J. Bone Jt. Surg.* **12**, 827-844.
- LOESCHKE, H. & WEINNOLD, H. (1922). Über den Einfluss von Druck und Entspannung auf das Knochenwachstum des Hirnschädels. *Beitr. path. Anat.* **70**, 406-439.
- MAIR, R. (1926). Untersuchungen über die Struktur der Schädelknochen. *Z. mikr.-anat. Forsch.* **5**, 625-667.
- MASSLER, M. & SCHOUR, I. (1951). The growth pattern of the cranial vault in the albino rat as measured by vital staining with alizarin red 'S'. *Anat. Rec.* **110**, 83-101.
- VON MIJSBERG, W. A. (1932). Die Funktion der Nähte am wachsenden Schädel. *Z. Morph. Anthr.* **30**, 535-551.
- MOSS, M. L. (1954). Growth of the calvaria in the rat. *Amer. J. Anat.* **94**, 333-362.
- PETERSEN, H. (1930). Die Organe des Skelettsystems. In Von Möllendorff's *Handbuch der mikroskopischen Anatomie des Menschen*, **2**, II. Berlin: Springer.
- PRITCHARD, J. J. (1946). Repair of fractures of the parietal bone in the rat. *J. Anat., Lond.*, **80**, 55-60.
- PRITCHARD, J. J. (1952). A cytological and histochemical study of bone and cartilage formation in the rat. *J. Anat., Lond.*, 259-277.
- PRITCHARD, J. J. & RUZICKA, A. J. (1950). Comparison of fracture repair in the frog, lizard and rat. *J. Anat., Lond.*, **84**, 236-261.
- SCOTT, J. H. (1954). The growth of the human face. *Proc. R. Soc. Med.* **47**, 91-100.
- SITSEN, A. E. (1933). Zur Entwicklung der Nähte des Schädeldaches. *Z. ges. Anat., Z. Anat. Entw-Gesch.* **101**, 121-152.
- SYMONS, N. B. B. (1952). The development of the human mandibular joint. *J. Anat., Lond.*, **86**, 326-332.
- TROITSKY, W. (1932). Zur Frage der Formbildung des Schädeldaches. *Z. Morph. Anthr.* **30**, 504-532.
- WASHBURN, S. L. (1947). The relation of the temporal muscle to the form of the skull. *Anat. Rec.* **99**, 239-248.
- WEIDENREICH, F. (1930). Das Knochengewebe. In Von Möllendorff's *Handbuch der mikroskopischen Anatomie des Menschen*, **2**, II. Berlin: Springer.
- WEINMAN, J. P. & SICHER, H. (1947). *Bone and Bones. Fundamentals of Bone Biology*. London: H. Kimpton.
- WEINNOLD, H. (1922). Untersuchungen über das Wachstum des Schädels unter physiologischen und pathologischen Verhältnissen. *Beitr. path. Anat.* **70**, 311-341, 345-391.

EXPLANATION OF PLATES

PLATE 1

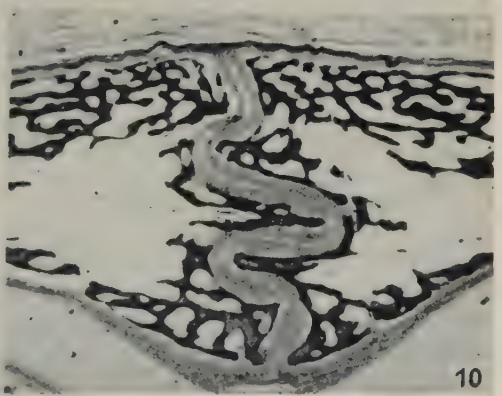
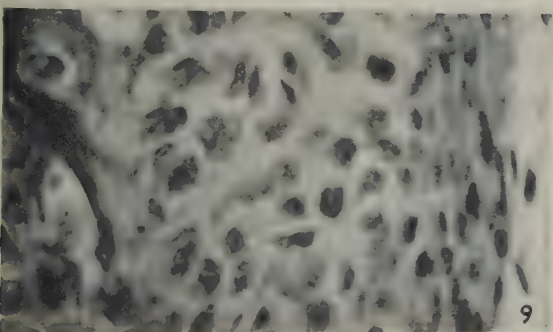
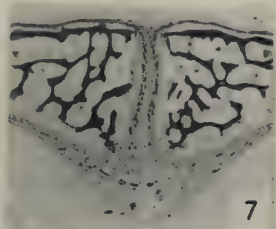
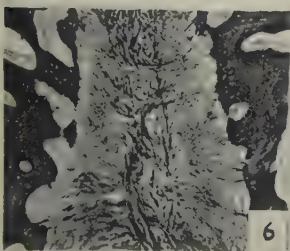
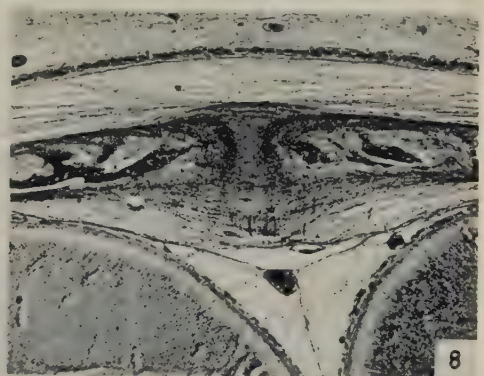
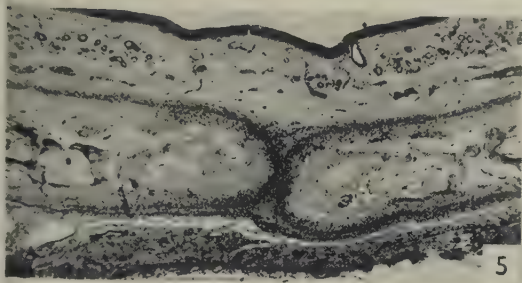
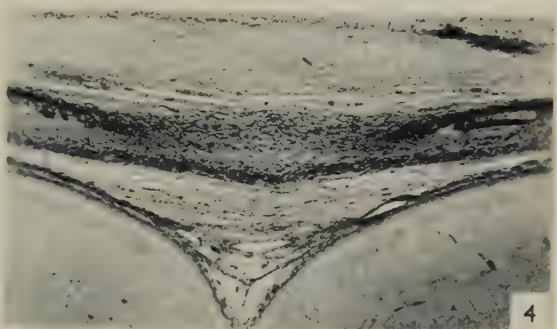
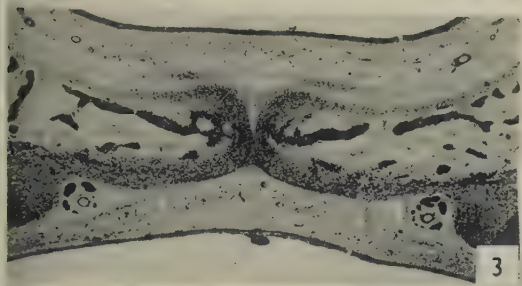
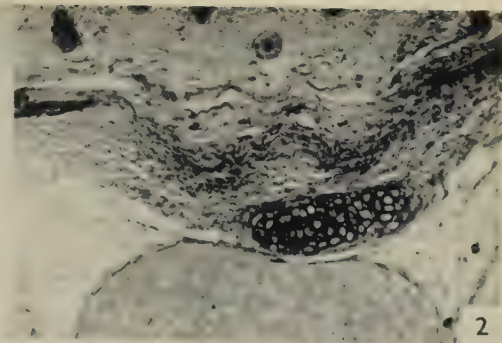
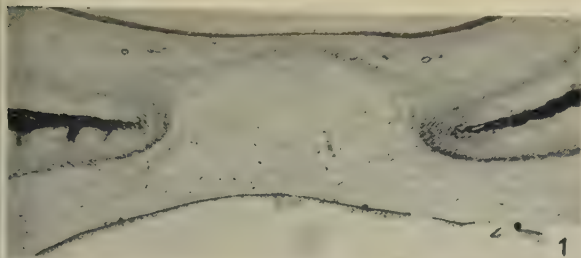
- Fig. 1. 75 mm. C.R. sheep foetus. Presumptive palatal suture. Weigert's haematoxylin and Van Gieson. $\times 32$.
- Fig. 2. 20-day rat foetus. Presumptive sagittal suture. Shows tectal cartilage. Haematoxylin and safranin. $\times 100$.
- Fig. 3. 90 mm. C.R. sheep foetus. Palatal suture. Shows meeting of bone territories. Weigert's haematoxylin and Van Gieson. $\times 25$.
- Fig. 4. 90 mm. C.R. sheep foetus. Presumptive sagittal suture. Shows beginning of delamination of ectomeninx at the site of the future suture. Wilder. $\times 25$.
- Fig. 5. 110 mm. C.R. sheep foetus. Palatal suture. Shows formation of uniting layers. Mallory. $\times 25$.
- Fig. 6. 9 months' postnatal sheep. Nasal suture. Shows Sharpey's fibres. Mallory. $\times 43$.
- Fig. 7. 125 mm. C.R. pig foetus. Nasal suture. Shows capsular and uniting layers. Masson. $\times 25$.
- Fig. 8. 110 mm. sheep foetus. Sagittal suture. Shows early formation of capsular layers. Wilder. $\times 25$.
- Fig. 9. 75 mm. C.R. sheep foetus. Edge of bone territory in palate showing cellular lamination. Weigert's haematoxylin and Van Gieson. $\times 420$.
- Fig. 10. Newborn pig. Nasal suture. Masson. $\times 25$.

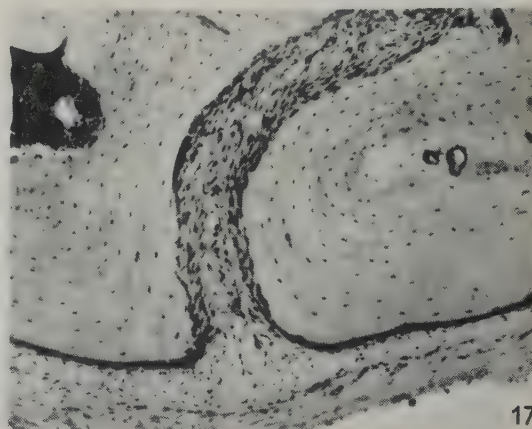
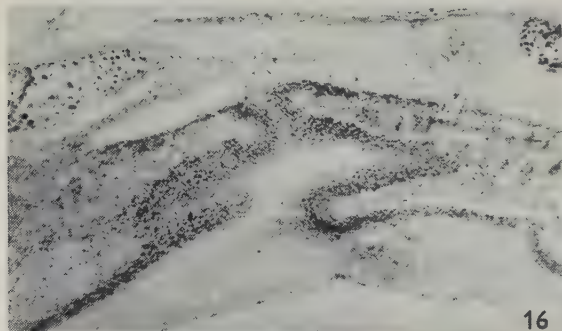
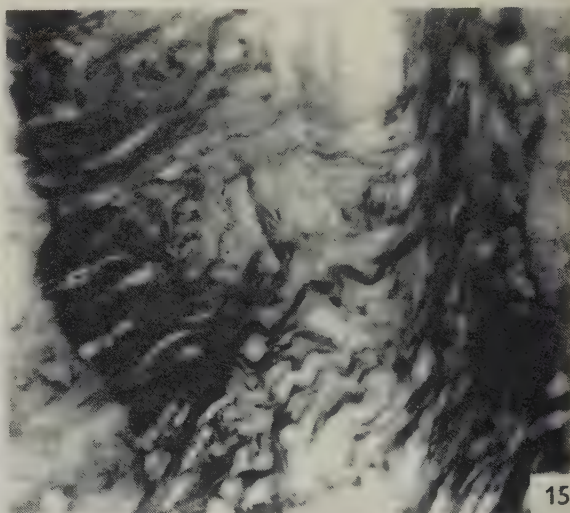
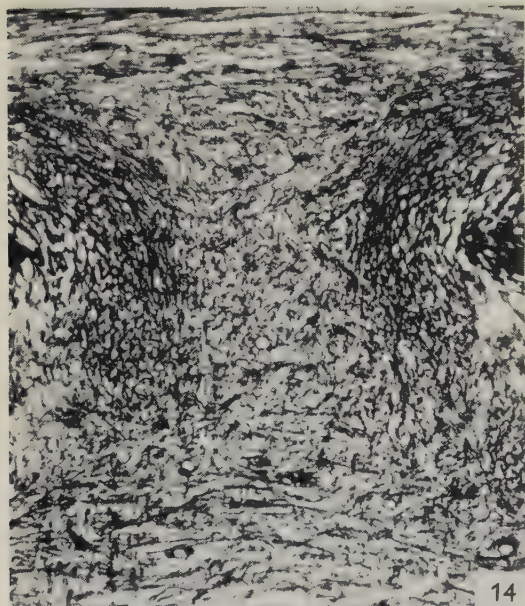
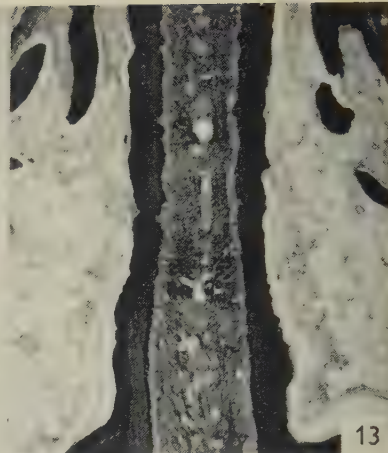
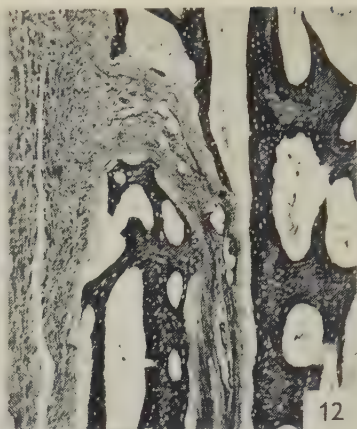
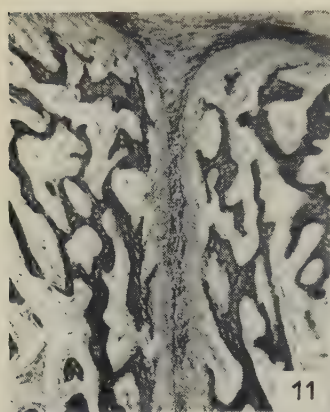
PLATE 2

- Fig. 11. 250 mm. C.R. sheep foetus. Palatal suture. Early growing stage. Masson. $\times 43$.
- Fig. 12. Newborn sheep. Part of sagittal suture. Late growing stage. Wilder. $\times 43$.
- Fig. 13. 57-day-old cat. Nasal suture. Adult stage. Mallory. $\times 43$.
- Fig. 14. 110 mm. C.R. sheep foetus. Sagittal suture. Higher power view of part of Fig. 8. Shows arrangement of fibres in capsular, middle and uniting layers. Wilder. $\times 150$.
- Fig. 15. Adult rabbit. Nasal suture. Shows capsular and middle layer fibre pattern. Mallory. $\times 420$.
- Fig. 16. Newborn rat. Palato-maxillary suture. Glycogen shown by periodic-acid-Schiff method. $\times 43$.
- Fig. 17. Adult rat. Part of sagittal suture showing cellular structure. Haematoxylin and eosin. $\times 150$.

PLATE 3

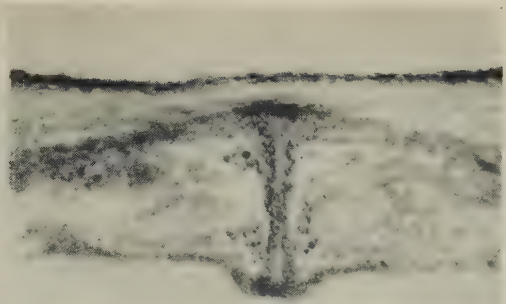
- Fig. 18. Newborn rat. Palatal suture. Alkaline phosphatase shown by Gomori's method. $\times 100$.
- Fig. 19. Newborn rat. Palatal suture. Glycogen shown by periodic-acid-Schiff method. $\times 100$.
- Fig. 20. 6-day-old rat. Palatal suture. Cambial layers replaced by cartilage. Haematoxylin and eosin. $\times 110$.
- Fig. 21. 14-day-old rat. Sagittal suture. Shows cartilage uniting bones across suture. Weigert's haematoxylin and Van Gieson. $\times 150$.
- Fig. 22. 3-day-old rat. Palatal suture. Shows synostosis with islands of hypertrophic cartilage. Masson. $\times 170$.
- Fig. 23. Adult rat. Temporo-parietal suture. Shows great vascularity of middle layer. Haematoxylin and eosin. $\times 100$.
- Fig. 24. 3-week-old rat. Sagittal suture. Shows partial synostosis. Weigert's haematoxylin and Van Gieson. $\times 150$.



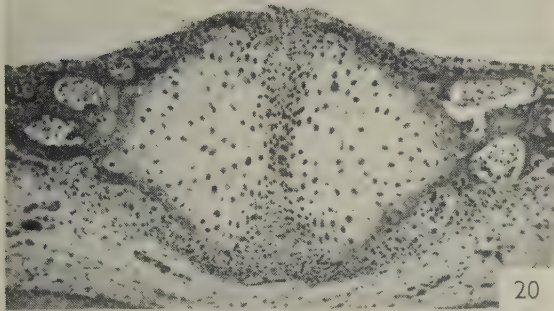




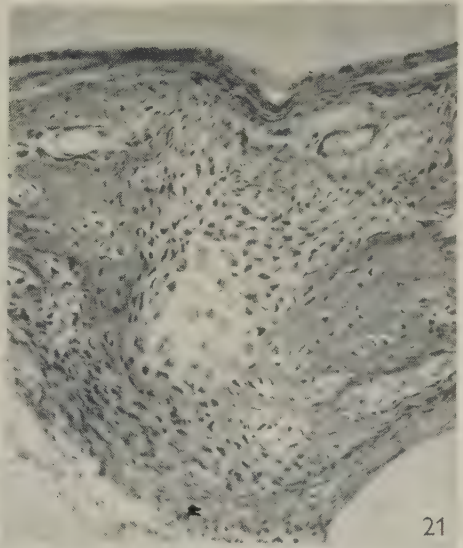
18



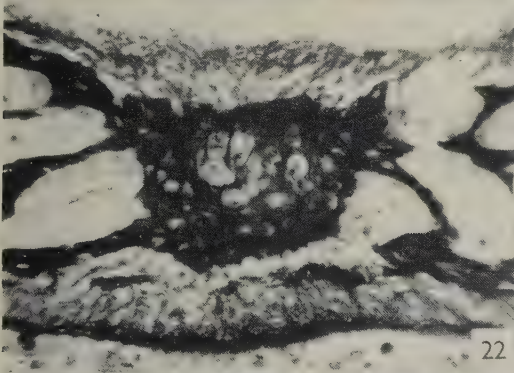
19



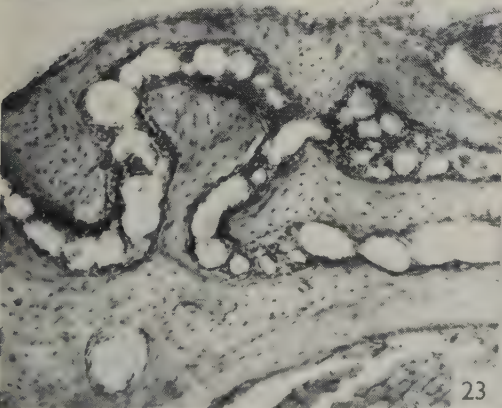
20



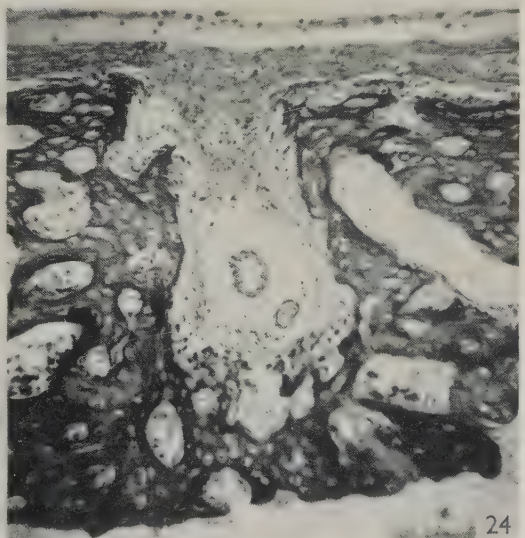
21



22



23



24

THE RELATIONSHIP BETWEEN THE PATTERN OF OSSIFICATION AND THE DEFINITIVE SHAPE OF THE MESOSTERNUM IN MAN

By G. T. ASHLEY

Anatomy Department, University of Manchester

INTRODUCTION

Variations in the number of bones forming the human mesosternum were noted by many of the old anatomists (Coiter, 1573; Kerckring, 1685; Eustachius, 1714); and the existence of a relationship between the manner of ossification and the definitive shape of the mesosternum has been hinted at by several observers, although the exact nature of this relationship has never been investigated. Kerckring stated, 'cum in sterni ossificatione nusquam natura ipsa constet, non mirum est autores ita variare, ut nemo non alteri contradicat'. Tarin (1753) recognized that there are inconsistencies, not only in the times of appearance of the various ossification centres, but also in the size attained by each centre. His comment on this point was as follows: 'Ils (the centres) paroissent dans quelques parties du sternum et s'y soudent suivant certaines loix; ils ne laissent pas que de s'en écarter quelquefois, et même assez considérablement, et comme le sternum ne commence pas toujours son ossification dans le même tem(p)s, il varie aussi dans son accroissement et pour son accomplissement.'

Frey (1935) says, translated, 'an essential supplement to our study will be the development of the form of the infant sternum and its relation to the process of ossification; this study is now in process of investigation and will, as far as we can see at present, fit in smoothly with the opinion we have already gained of the forms in which the human sternum is found': unfortunately she died a few years later, and I can find no record of a further communication having been published.

Schultz (1944) states: 'the ossification of the sternum of Gibbons is an exceedingly variable process and, most likely on this account, the conformation and shape of the adult sternum are hardly ever alike in any two Gibbons'.

Particular attention has been paid to the *pattern* of ossification in the developing sternum, by Paterson (1904), Markowski (1902, 1905) and Herdner (1947). However, these observers did not relate the patterns of ossification discovered to the various shapes which one may observe in adult bones. Further, there was little agreement between the conclusions reached by Paterson and Markowski.

In 1951 and 1953 I put forward evidence to show that a very definite relationship does exist between the shape of the adult mesosternum and the number and arrangement of the ossification centres from which the bone develops. A particular ossification pattern is generally associated with a particular shape. In order to ascertain the *fundamental* cause of the variation in the shape of the mesosternum it is necessary to ascertain the cause of the variability in ossification pattern which leads to the variability in definitive shape.

At the same time it will be convenient to consider the aetiology of the abnormality known as sternal foramen. Eustachius (1707) claimed to be the first to describe this condition, but according to Riolanus (1649) a foramen in the mesosternum was first observed by Massa. From the description given by Riolanus, it is obvious that Massa considered perforation of the mesosternum to be a feminine characteristic, believing that the foramen exists for the transmission of a mammary vein. This conception was reiterated by Rhead (1634) who writes in the picturesque language of his time: 'The breast-bone (is) perforated sometimes with a hole much like the forme of a heart, through which veines doe runne outward from the mammary veines unto the paps.'

Crooke (1615) and Bartholin (1668) attached similar significance to a foramen in the xiphoid process, the latter observer saying, 'sometimes 'tis perforated for the Dug-veins and arteries, which are accompanied by a nerve'. Diemerbroeck (1679, 1685) agreed with Massa. Blumenbach (1786) stated (translated): 'Sometimes, but quite rarely, one finds the lower end of the sternum bored through by a hole, which varies in position as well as in size and is probably developed by mere accident when neighbouring bone nuclei fail to meet completely. The old idea that this hole appears more frequently in the female than in the male is unfounded.' Broc (1836) agreed that the condition is not influenced by sex. Monro (primus) (1758) and Cloquet (1828) supported the view that the foramen results from defective ossification and, more recently, Lickley (1904), Greig (1926) and Grant (1944) have concurred with this view. Paterson (1904) seemed to be undecided about the aetiology of sternal foramen. He states, on p. 43 of his monograph: 'One rejects the idea of sternal foramina being associated with the occurrence of fissura sterni; the causes of the two conditions seem to be distinctly different.' Yet on p. 19 of the same work he states: 'grooving of the mesosternum is probably associated with the method of early development of the cartilage....Like the grooves...foramina are not improbably causally associated with the mode of early development of the sternum.' This being true it is difficult to see how either sternal foramen or sternal grooving can causally be dissociated from sternal fissure, except in regard to the *degree* of maldevelopment.

Early stages in the development of the sternum were described by Rathke (1838), and in greater detail by Ruge (1880). Malgaigne (1859) stated his belief that the presence of a sternal foramen indicated 'un arrêt dans la conjugaison de ses deux moitiés laterales chez l'embryon'. The fundamental truth of this statement became widely accepted, most anatomists agreeing that sternal foramen indicates arrest of sternal development. They agree also that the arrest in development is at a stage somewhat advanced beyond the state which, very rarely, persists as sternal fissure (Luschka, 1863; Förster, 1865; Pansch, 1875; Gibson & Malet, 1879; Debierre, 1890; Merkel, 1899; Keith, 1948; Gray, 29th English edn.). Förster stated the case clearly and concisely, as follows: 'Als geringster Grad der Spaltung kann derjenige Fall angesehen werden, in welchen sich in der Mittellinie des Sternum ein oder mehrere ovale Löcher finden.'

In view of the weight of evidence, it is apparent that the presence or absence of a sternal foramen is determined *in the pre-ossification stage of development*, i.e. when the sternum is still cartilaginous. Therefore, 'defective ossification' is the *result* not the *cause* of a sternal foramen.

The incidence of mesosternal foramen has been variously estimated by different observers, viz. Bogusat (1902) 2.5 %; Paterson (1904) 3.8 %; Meriel (according to Greig (1926)) 5.7 %; Stieve & Hintzsche (1925) 6.25 % in Germans, 15.4 % in Papuans; Schultz (1944) 12.4 % in negroes.

The series of sterna which I have examined included numerous specimens presenting a foramen in the mesosternum. As will be shown, these specimens were found to be of a distinctive and significant shape.

The review of the literature has revealed that certain points of controversy still exist concerning the true significance of variability in the shape of the mesosternum. In the present investigation I have attempted to ascertain the precise relationship which exists between variability in (a) the manner of ossification and (b) the definitive shape of the mesosternum.

MATERIAL AND METHODS OF STUDY

The material studied and the methods of study are indicated in Table 1.

Table 1. *Summary of material studied and methods of study*

	Inspection	Measurement	Radiography	Histological section
Adult sterna				
African	118	98	98	0
European	676*	573	585	0
Immature sterna (age 2-19 years)				
African	7	—	7	0
European	133*	—	123	0
Infant sterna (birth-1 year)				
European	73*	—	71	0
Foetal sterna (10 weeks-full term)				
European	393*	—	166	50
Whole embryos (4-40 mm.) (4-9 weeks)	10	—	—	10

* Including many specimens from the Paterson collection at Liverpool University.

Inspection

Inspection involved observance of general shape and of foramina, notches, grooves, ridges, etc., and abnormalities if present.

Measurements

Measurements taken were as indicated in Text-fig. 1. All measurements are recorded to the nearest millimetre.

From these measurements the following proportions were ascertained in the case of adult specimens:

The Manubrium-Corpus Index, i.e.

$$\text{Manubrial length as a percentage of mesosternal length} = \frac{M \times 100}{B}.$$

The Mesosternal Relative Width Index, i.e.

Width of first mesosternal segment as a percentage of

$$\text{width of third mesosternal segment} = \frac{S_1 \times 100}{S_3}.$$

Radiography

Radiographs were taken by postero-anterior projection, as I have found that this method limits distortion of any 'fusion lines' which may be present. Such fusion lines in conjunction with foramina, grooves, notches, etc., were utilized, where possible, in assessing the ossification pattern of each mesosternum. The pattern was practically always apparent in foetal sterna; usually evident (80 % of cases) in infant and juvenile sterna; and frequently discernible (49 % of Europeans, and 65 % of Africans) in adult sterna, especially in the younger adult material.

FINDINGS

The findings of the investigation are recorded and discussed under the following headings:

I. Ossification patterns in immature sterna—four basic types.

II. Ossification patterns in adult sterna (*a*) in Europeans, (*b*), in East Africans—with a note on the relation of sternal foramen to the pattern of ossification.

III. The 'properties' of adult sterna associated with the four basic types of ossification (*a*) in Europeans, (*b*) in East Africans.

IV. The relation of ossification pattern and definitive shape (*a*) to sex irrespective of race, (*b*) to race irrespective of sex.

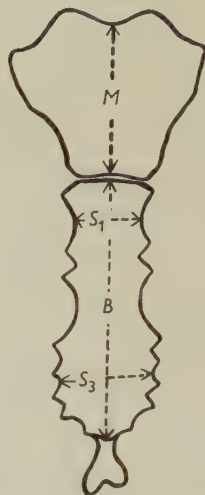
I. Ossification patterns in immature sterna—four basic patterns

Radiography of isolated immature sterna has revealed that although there is great variability in minor details, in general it may be said that the centres of ossification in the mesosternum appear in patterns which may be arranged in a more or less orderly manner. The plan which I have adopted for classifying the various patterns is shown in Text-fig. 2. For the sake of simplicity this plan covers only sterna in which the mesosternum consists of four or less segments. The occasional occurrence of a five-segment mesosternum does not affect the principle involved.

Four basic patterns

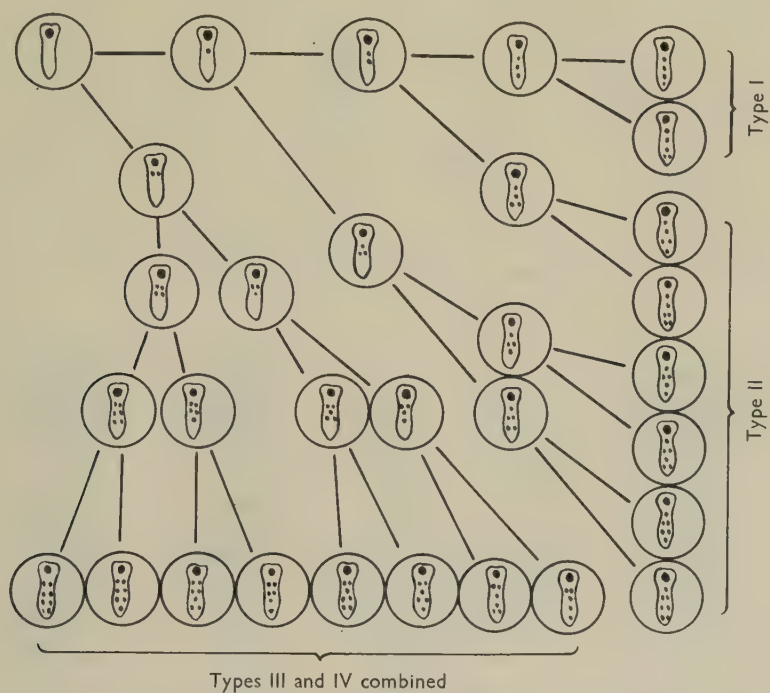
It is seen from Text-fig. 2 that a total of sixteen *regular* patterns is possible in mesosterna composed of four segments. Irregular patterns, which are common, can be classified with the regular patterns to which they most nearly conform. The sixteen regular patterns can be divided up into four groups in such a way as to form four basic patterns which may be described as follows:

Ossification pattern type I. In each of the first three segments of the mesosternum



Text-fig. 1. Measurements taken: *M*, length of manubrium in mid-line; *B*, greatest length of body of sternum (i.e. mesosternum); *S*₁, width at waist of first segment of mesosternum; *S*₃, width at waist of third segment of mesosternum.

the ossification centres are usually single and always mid-line. Occasionally they may be double *vertically*, but still, *strictly mid-line in position*. In the fourth segment centre(s) may be single, double or completely absent (see Text-fig. 2).



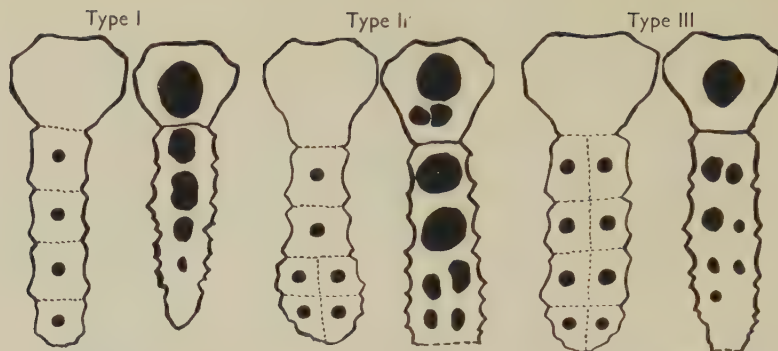
Text-fig. 2. This plan shows most of the variations which may be observed in the ossification pattern of the mesosternum at various ages—and indicates how the author has grouped these variations into 'types'.

Ossification pattern type II. In either the first or first and second segment(s) of the mesosternum the ossification centre(s) is/are single and mid-line, whereas in the second (in some), third (in all) and fourth (in some) the centres are double, and bilaterally or obliquely placed (see Text-fig. 2).

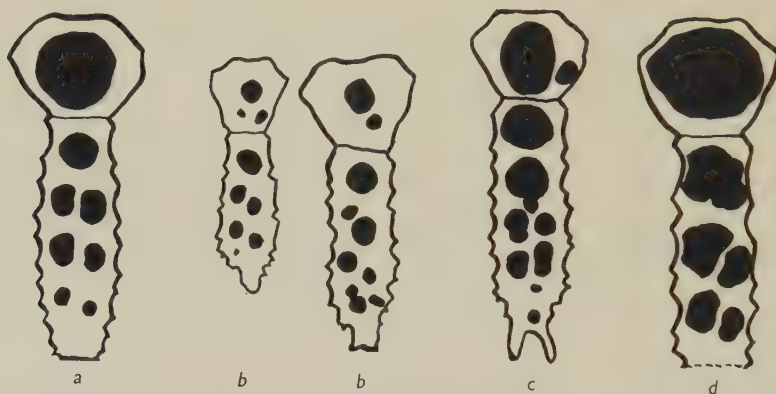
Ossification pattern type III. In each of the first three segments of the mesosternum the ossification centres are double (bilaterally or obliquely), and in the fourth segment the centre(s) may be double, single or absent (see Text-fig. 2).

Ossification pattern type IV. In either the first or first and second segment(s) the centres are double, bilaterally or obliquely; whereas in the third they are single and in the fourth single or absent. This pattern is very rarely encountered in post-natal human sterna, and, for further simplification, I have included the few specimens observed with type III.

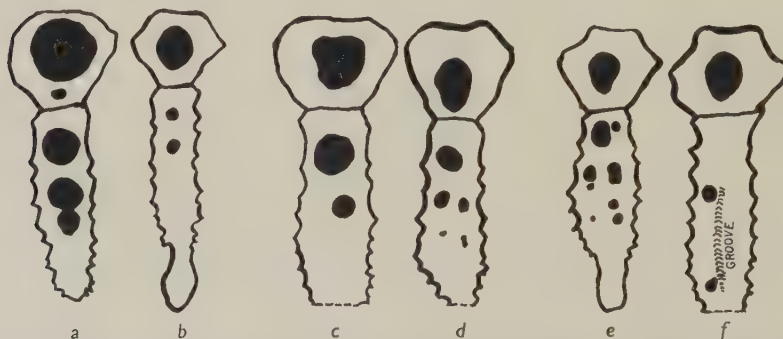
In Text-fig. 3 the 'ideal' patterns in types I–III are shown, together with radiographs showing the 'typical' patterns. Other radiographs showing some of the many atypical variants are shown in Text-fig. 4.



Text-fig. 3. 'Ideal' patterns, with tracings of radiographs of actual examples. These three patterns of ossification are utilized for classification purposes.



Text-fig. 4. Tracings of radiographs showing some of the numerous variations in pattern, indicating the manner in which such variants were classified: (a) the most common variant of type II; (b, b) obliquity of paired centres—both type II; (c) supernumerary centres—type II; (d) type III pattern in a 3-segment mesosternum.



Text-fig. 5. Determination of type of ossification pattern in immature foetal sterna. (a) type I; (b, c), indeterminate (may ultimately become either type I or type II); (d) type II; (e) type III; (f) indeterminate (may ultimately become either type II or type III).

It will be seen from Text-fig. 3 that these three types correspond respectively to what Markowski (1902) called single-row, single/double-row, and double-row patterns.

In order to determine the incidence of these three basic patterns of ossification, I have studied 596 immature sterna, in 581 of which the age was known to the nearest month.

I have examined each specimen with the following queries in mind:

(i) Are sufficient ossification centres present to permit of classification according to the plan shown in Text-figs. 2 and 3?

If not, then discard the specimens from further consideration (see Text-fig. 5*b*, *c* and *f*).

(ii) Has the pattern of ossification been obscured by complete synostosis between individual centres?

If so, then discard.

(iii) From the number and arrangement of centres present in specimens not discarded in accordance with (i), (ii) above, does the 'pattern' suggest types I, II or III?

On this basis, from the 581 immature sterna examined 143 specimens were discarded (117 with pattern indeterminate, 26 with pattern obscured). Details of the 581 specimens are shown in Table 2, and a further analysis of the specimens 'typed' is shown in Table 3 and Text-fig. 6.

Table 2. *Classification of ossification patterns in 581 immature sterna*
(age known to nearest month)

Age	No. of specimens	Typed according to plan			Discarded because pattern	
		Type I	Type II	Type III	(a) Indeterminate (proportion diminishing)	(b) Obscured (proportion increasing)
Foetal						
5-6 m.	71	18	8	2	43	0
7-8 m.	70	12	19	7	32	0
9 m.	216	64	83	34	35	0
Post-natal						
0-1 yr.	78	22	43	7	6	0
1-4 yr.	37	11	21	3	1	1
5-9 yr.	27	5	17	3	0	2
10-14 yr.	26	6	11	2	0	7
15-19 yr.	56	12	25	3	0	16
Total	581	150	227	61	117	26
No knowledge of age	15	Total number 'Typed' 438			Total discarded 143	
Total	596	See Table 3 and Text-fig. 6 for further analysis			Not further considered	

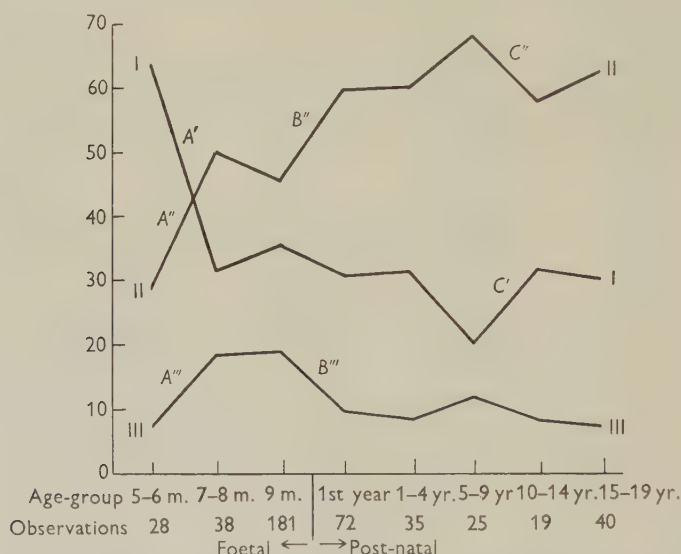
From Text-fig. 6 it is seen that during the 5th and 6th foetal months the majority of sterna (more than 60%) appear to be developing from single mid-line centres. This appearance is due to the fact that both centres of a 'pair' rarely appear simultaneously (see Pl. 1, fig. 1).

As additional centres are added during the 7th and 8th months some of the apparent type I sterna become converted into either type II or type III sterna. The

Table 3. *Further analysis of the 438 immature sterna in which the ossification pattern could be determined (see Table 2)*

Age	No. of specimens	Typing according to plan (Text-figs. 2-5)					
		Type I		Type II		Type III	
		No.	%	No.	%	No.	%
Foetal							
5-6 m.	28	18	64.3	8	28.6	2	7.2
7-8 m.	38	12	31.6	19	50.0	7	18.4
9 m.	181	64	35.4	83	45.8	34	18.8
Post-natal							
0-1 yr.	72	22	30.6	43	59.7	7	9.7
1-4 yr.	35	11	31.4	21	60.0	3	8.6
5-9 yr.	25	5	20.0	17	68.0	3	12.0
10-14 yr.	19	6	31.6	11	57.9	2	8.5
15-19 yr.	40	12	30.0	25	62.5	3	7.5
Total	438						

The percentages shown above are recorded graphically in Text-fig. 6



Text-fig. 6. Graphs prepared to show the change in the proportions of *apparent* types I-III patterns of ossification at different ages. Reasons for the various changes indicated are elucidated in the text.

graph in Text-fig. 6 illustrates this point. The decline in the proportion of type I (A' in line I) is accompanied by increase in the proportion of types II and III (A'' on line II and A''' on line III).

In the 8th and 9th months the pattern of ossification is of type III in 18%. Whereas, in some of these, the right and left columns of centres are widely spaced throughout, as in Pl. 1, fig. 2A, in others the paired centres in the *first* mesosternal segment are very close together, as in Pl. 1, fig. 2B. In the latter case, the pair of centres rapidly coalesce to form, apparently, one median centre, and the 'pattern' changes to type II (Pl. 1, fig. 2C).

The above fact, also, is indicated in the graph (Text-fig. 6). The fall in the proportion of type III and the accompanying rise in the proportion of type II during the first post-natal year are indicated by *B'''* in line III and *B''* in line II respectively.

In a similar manner one occasionally finds that some of the paired centres in sterna of type II are, from the outset, closely approximated. Such pairs of centres may coalesce during childhood and convert these specimens into apparent type I sterna (see Pl. 1, fig. 3).

Once again, the change is revealed in the graph (Text-fig. 6). The decrease in the proportion of type II sterna, during the period from the fifth to the tenth year, is associated with an increase in the proportion of (apparent) type I sterna (observe *C''* on line II and *C'* on line I).

It is obvious from the foregoing that the *real* ossification pattern can best be determined at the time when *most* of the centres are present as individual entities. This short period includes the last month of foetal life and the first 4 years of post-natal life. During this period sufficient centres have appeared for the 'pattern' to be determinable; and as yet, very few of the centres have so completely coalesced with their neighbours as to obscure the 'pattern' from the searching rays of the X-ray apparatus.

On this basis it is estimated, from Text-fig. 6, that about 60 % of human sterna develop from an ossification pattern of type II, about 22 % from a pattern of type I, and the remaining 18 % or so from a pattern of type III.

As will be shown later, in many cases traces of the ossification pattern persist until late in adult life. Furthermore, the proportions of types I-III to be found at any stage of life are not vastly different from the proportions found in childhood.

II. *Ossification patterns in adult sterna*

By visual observation alone one may, in some cases, obtain a limited amount of information concerning the number and arrangement of ossific elements which have fused together to form the mesosternum. One may utilize as evidence the following features: foramina (*vide infra*), clefts, notches, grooves, and ridges.

Much more conclusive evidence can be obtained from careful study of radiographs of the isolated sternum than from inspection alone. In addition to foramina, clefts, notches, etc., one may often observe lines of increased density where adjacent ossific elements have come together.

A note on the significance of a foramen in the mesosternum as an aid to 'typing' the sternum

In the East African series no less than thirteen out of a total of ninety-eight sterna presented a foramen in the mesosternum. The defect was always in the mid-line and always opposite, or nearly opposite, the fifth costal cartilages. Radiographs revealed that in nine of the thirteen cases the foramen was associated with the pattern of ossification which is characteristic of type II (see Pl. 2, fig. 6). In the remaining four cases the ossific pattern had become obliterated through age. It may be asserted with confidence that the mere presence of a foramen in the mesosternum indicates that such a mesosternum has ossified, in part at least from bilateral centres.

In addition to the ninety-eight East African sterna, I have examined radiographs of 573 European 'adult' sterna (i.e. from individuals aged 20 years or more). These were all of known age and sex. A mesosternal foramen was present in 4% of European sterna.

In the African series, radiography revealed the ossification pattern in sixty-four specimens (i.e. 65%).

In the European series, which included many aged specimens, radiography revealed the pattern in 283 specimens (i.e. 49%).

Considering only the sixty-four African and the 283 European adult sterna in which the pattern was discernible, the proportions of types I-III were as shown in Table 4. These figures are closely comparable to the proportions found in immature sterna (see Tables 2 and 3 and Text-fig. 6).

In other words, the patterns of childhood persist to adulthood in about 50-60% of individuals but become obscured in the remainder.

The 'ideal' ossification patterns of types I-III are revealed in the radiographs of selected adult specimens shown in Pl. 2, fig. 7.

III. The 'properties' of adult sterna associated with the four basic patterns of ossification *General observations concerning the shape of the mesosternum*

Ossification pattern type I. Adult sterna which, from radiographic evidence, had apparently developed from this pattern of ossification were found to be of a distinctive shape—the mesosternum being narrow and having more or less parallel sides (see Pl. 2, figs. 7, 8).

Ossification pattern type II. In sterna derived from this pattern of ossification the mesosternum is always narrow in its first segment and wide in its third (see Pl. 2, figs. 7, 8).

Ossification pattern type III. Here the mesosternum is found to be very wide throughout its length, having more or less parallel sides with the width of the first segment approximately equal to the width of the third (see Pl. 2, figs. 7, 8).

Ossification pattern type IV. This type of sternum is rarely encountered in man, but I have found it to be common in gorilla. In this type the mesosternum is wide in its first segment and narrow in its third and fourth segments. In the human series I have included such sterna with type III.

From the evidence provided by radiography I would say, categorically, that there is a fundamental relationship between the *pattern of ossification* and the *definitive shape* of the mesosternum. In view of this statement, it is necessary for me to stipulate, specifically, such absolute and relative measurements (or 'properties') as may prove of value in differentiating one type of sternum from another. Thereby it will become possible for subsequent observers to confirm or refute my views.

Specific measurements and ratios included under the term 'properties'

Measurements were first taken only from the particular adult sterna in which radiography had revealed the pattern of ossification (see Table 4). The measurements considered to be of value as criteria for differentiating one type of sternum from another were as follows:

(1) The width of the first mesosternal segment (S_1).

(2) The ratio $(S_1 \times 100)/S_3$ (see Text-figure 1). The ratio $(S_1 \times 100)/S_3$ gives an approximate idea as to how nearly 'parallel' are the sides of the sternum. A value of 100 indicates that the sides of the mesosternum are 'parallel'; a value of less than 100 that they diverge as one proceeds from first to third segment; a value of more than 100 that they converge as one proceeds from first to third segment.

Table 4. *Patterns of ossification discernible in adult sterna*

	Type I (%)	Type II (%)	Type III (%)
African	20.0	72.0	8.0
European	24.0	64.0	12.0

The absolute measurement (S_1) and the ratio $(S_1 \times 100)/S_3$ I have termed the 'properties' of the mesosternum.

The average 'properties' of the three basic types of sternum, in adult Europeans and Africans, were found to be as shown in Table 5, from which it will be seen that each 'type' fulfils two conditions, and in so doing is differentiated from the other two 'types'.

Table 5. *The 'properties' of the three types of sternum*

	Type I	Type II	Type III
Europeans S_1	Less than 29 mm.	Variable (16-34) but generally less than 29 mm.	29 mm. or more
$\frac{S_1 \times 100}{S_3}$	More than 85	Less than 85	More than 85
Africans S_1	Less than 28 mm.	Variable (17-28) but generally less than 28 mm.	28 mm. or more
$\frac{S_1 \times 100}{S_3}$	More than 85	Less than 85	More than 85

This method of classifying sterna is entirely original. It is considered that the results obtained reveal that the method is based on sound morphological principles.

Certain observers (Lubosch, 1920; Stieve & Hintzsche, 1925) have used the length and breadth index devised by Martin (1914). I have not used this index as it is obvious from the works quoted that it is a ratio of dubious morphological value.

It is stressed that the 'properties' above defined were obtained from measurements of 283 adult European and sixty-four adult African sterna in all of which the ossification pattern had been revealed by radiography.

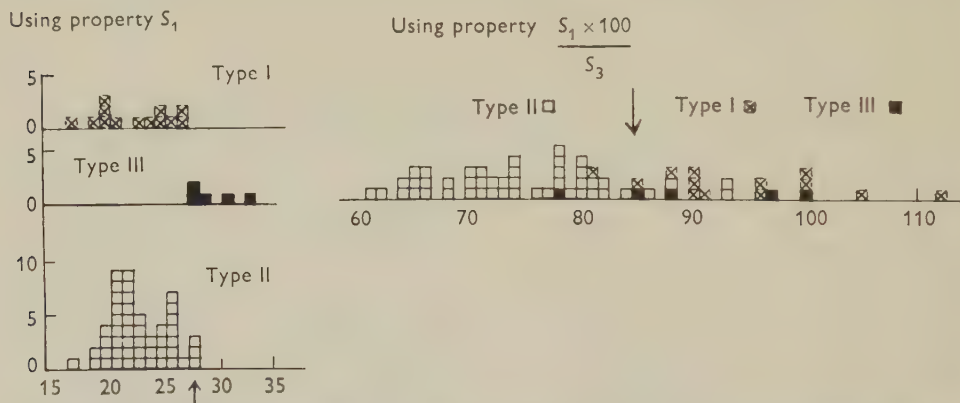
To illustrate the value of these 'properties' as differentiating features, I have prepared the graphs shown in Text-fig. 7. From these it may be seen that of sixty-four adult African sterna in which the pattern of ossification was clearly evident, only six (9.4 %) failed to comply with the requirements stated in Table 5. The 283 European sterna were tested in a similar manner and only thirty-three (11.8 %) did not possess the requisite shape 'properties' applicable to the pattern revealed by radiography.

I then measured the 290 European and the thirty-four African adult sterna in which radiography had failed to reveal the ossification pattern owing to the absolute synostosis of the individual elements of the mesosternum.

Employing the measurements listed in Table 5, I arbitrarily divided these sterna, in which the patterns were obscured, into three groups according to the type 'properties' applicable to the sterna in which the patterns were still apparent. I found the three types in the following proportions:

	European (%)	African (%)
With 'properties' of type I	25.0	20.0
With 'properties' of type II	65.0	73.0
With 'properties' of type III	10.0	7.0

Again, these figures are comparable with the proportions previously recorded for sterna, of various ages, in which radiography had revealed the pattern of ossification (see Tables 3 and 4 and Text-fig. 6). The obvious inference to be drawn from this



Text-fig. 7. Illustrating how the 'properties' described in the text are used in differentiating the three types of sternum in adults. Arrows indicate the critical values.

finding is that one can, by simple measurement, ascertain the probable ossification pattern of any particular adult human sternum. As will be revealed in the Discussion, there are exceptions to this, as to any other, biological generalization. Nevertheless, there can be no doubt about the general principle which links together the ossification pattern and the definitive shape of the sternum. Having established this principle, it is of interest to record the relationship which exists between sex, race and the proportions of the sternal types described.

IV. The relation of ossification pattern and definitive shape (a) to sex irrespective of race, (b) to race irrespective of sex

(a) Relation to sex irrespective of race

The proportions of the three types of sternum found in males and females at various ages are summarized in Table 6.

Table 6. *The proportions of the three types of sternum found in males and females at various ages*

Age	Type I (%)		Type II (%)		Type III (%)	
	M.	F.	M.	F.	M.	F.
Full-term foetal and infant	26.7	38.7	55.6	51.6	17.7	9.6
Child and adolescent (1-19)	22.0	33.0	70.0	60.0	8.0	7.0
Adult	22.2	26.2	66.4	61.2	11.4	12.6

(b) *Relation to race irrespective of sex*

The proportions of the three types of sternum found in adult Europeans and East Africans were shown in Table 4.

In the absence of corroborative evidence from other workers, no attempt is made to attach any special statistical significance to such difference as exists between (a) the sexes, and (b) the two races considered. More significant sexual differences have been reported elsewhere (Ashley, 1955). It would appear, however, that the type I sternum is found more frequently in females than in males. This is surprising, in view of the relatively greater width of the lower part of the mesosternum remarked on by Paterson and confirmed in the investigation referred to (Ashley, 1955).

DISCUSSION

As some of the findings reported above are either new, or at variance with accepted teaching, the ensuing discussion will be concerned with comparing my own views with those of previous workers. Fundamental to the whole problem concerning the variability in the shape of the sternum is the question of the manner of ossification of the bone. Therefore, this problem will be discussed first.

Concerning the ossification patterns in immature sterna

My findings concerning the proportions of the three basic patterns of ossification in full-term foetal, infant and juvenile sterna closely resemble the findings of Markowski, but differ from the findings of Paterson as is evident from Table 7.

Table 7. *Patterns of ossification in immature sterna*

Observer	Age group	Type I (%)	Type II (%)	Type III (%)
Paterson (1904) (estimated from his Table 2)	4-9 m. foetal	59.0	20.0	21.0
	0-16 y.	65.5	31.0	3.5
Markowski (1905)	Foetal and infant	33.0	57.0	10.0
(Author) European sterna	Full-term foetal	35.4	45.8	18.8
	Infant	30.6	59.7	9.7

The similarity between my findings and those of Markowski is quite striking. At first sight it is difficult to reconcile these findings with those deduced from figures given in Paterson's monograph. The discrepancy is all the more remarkable when

it is recalled that details of the ossification pattern in about two-thirds of the sterna examined by me were obtained either from specimens in the Paterson collection, or from Prof. Paterson's manuscript records. This paradoxical situation has undoubtedly arisen because Paterson, in compiling his tables, combined all of his foetal sterna into one group, including sterna from very young foetuses in which the ossification pattern was as yet incomplete. Furthermore, he did not use radiography to examine his post-natal specimens, and, therefore, was hardly in a position to pronounce, with any degree of certainty, on the pattern of ossification in such specimens. It is mainly for these reasons that his interpretation of the patterns of ossification differs so much from my own findings. As Tarin (1753) pointed out, and Markowski stressed, if two centres are destined to appear in any one segment they need not necessarily make their appearance simultaneously. Indeed one of them may attain quite large size before the second appears at all. The radiographs of foetal sterna shown in Pl. 1, fig. 1, confirm this. Markowski argued that if, in any segment of a foetal mesosternum, only one ossification centre is visible *and this centre is laterally placed*, then one is justified in assuming that the centre for the opposite side of that segment has not yet appeared. This is a logical assumption and it led Markowski to the conclusions quoted above. Nevertheless, I have not based my own conclusions on his deductive method. I have reached results comparable to the findings of Markowski by the technique described above, which is based not on assumption but on facts.

*A note on the probable cause of variability in the ossification pattern
of the mesosternum*

There must be some specific reason why some mesosterna ossify from single, median centres, whereas others ossify, in part or entirely, from double rows of centres.

I consider that the variability in the pattern of ossification is a result of inco-ordination between (a) the time and completeness of mid-line fusion of the lateral sternal bands, and (b) the times of appearance of the individual ossification centres. It seems logical to argue that if the two sternal bands have grown together *completely* before conditions are suitable for ossification to begin, then it is likely that ossification centres will be single and median. If, on the other hand, the two lateral bands fail, or partly fail, to grow together before ossification begins, then, in the part incompletely fused, it is likely that ossification centres will be paired and bilateral. Longitudinal mid-line grooving is to be seen on the anterior and/or posterior aspect of the mesosternum in a large percentage of sterna during the latter half of pre-natal growth. Such grooving is an indication that the lateral sternal bands have not completely grown together. Paterson found grooving in the whole or part of the length of the mesosternum in 70.1 % of 222 foetal sterna aged from 4 months to full-term. He stated: 'This grooving of the mesosternum is probably associated with the method of early development of the cartilage, *but it is at the same time plain that it is not necessarily coincident with a bilateral ossification of the mesosternum.*'

The assertion which I have shown in italics is, of course, completely at variance

with the hypothesis which I have just put forward. Accordingly, I have re-examined Paterson's detailed and individually illustrated manuscript records concerning these sterna, and it is apparent that Paterson relied too much on observed facts and too little on imagination. He failed to make allowance for the fact that in many of his foetal sterna the time was not ripe to state what the final ossification pattern would be. Had he made this allowance, he would have realized that in practically every case where grooving was present, all ossification centres *in relation to the groove* were laterally placed. In such cases it was still conceivable that when the full complement of centres had appeared the pattern would have included bilateral centres in, at least, the portion of the mesosternum where grooving was most marked.

In my own series of foetal sterna I have frequently noticed such vertical grooving of either the anterior or the posterior aspects of the sternum, usually the latter. It is often indicated on radiographs, since the cartilage of the sternum is thin in the region of the groove and therefore less resistant to the passage of X-rays. In Pl. 1, fig. 4A, B, are shown two foetal sterna each presenting unpaired ossification centres in the mesosternum. These might be mistaken for single mid-line centres, but on careful inspection it may be seen that in each case a mid-line groove is indicated. The centres in relation to this groove are to one or other side of the mid-line. In such cases it is probable that the other member of each 'pair' has not yet appeared to convert, for example, specimen A into a type II sternum, and specimen B into a type III sternum. In the third specimen shown (Pl. 1, fig. 4C) the vertical line does not indicate a natural groove, but resulted from an ill-judged incision by the mortician.

Concerning the ossification patterns in adult sterna and the shape of the mesosternum

I believe that in the majority of instances, say 90 %, one can with certainty place any given sternum into its appropriate group according to the method of 'typing' elaborated above. However, it must be recorded that intermediate types are found in the remaining cases. In such specimens the shape 'properties' obtained by measurement do not conform to the *observed* ossification pattern. For example, a sternum with a characteristic type II ossification pattern revealed by radiography, occasionally presents shape 'properties' which fall into the range of type I or vice versa. This detracts to a small degree from the statistical value of the method but does not deny the underlying principle. Possible reasons for some of the discrepancies have already been revealed (see Pl. 1, figs. 1-3). Furthermore, in study of a large series of foetal sterna it is inevitable that one occasionally encounters gross irregularity in either the pattern of ossification, or the time of appearance and rate of growth of the individual ossification centres. Nevertheless, every variation in ossification pattern in young sterna seems to have its counterpart in corresponding variations in the shape of adult sterna as, for example, the oddities shown in Pl. 1, fig. 5, where a pattern such as A or B may have led to the development of C. Far from disproving the hypothesis put forward, these variants lend support to the general principle that a particular form of mesosternum has a particular developmental history.

Herdner (1947) has been able to demonstrate the pattern of the ossification centres

in the mesosternum by tomographic examination in children. He has suggested that bilateral centres represent an early stage in development, and that the centres of each pair fuse together to form single median centres, unless prevented from so doing by illness or dietary deficiency.

Such generalization is not justified by facts elucidated in the present study, from which it is obvious that in the majority of sterna the pattern of ossification is of either type I or type II from its inception; that never more than 18–19 % of human sterna originate from completely bilateral sets of centres; and that the original patterns of ossification persist to adulthood in certainly more than 50 % of individuals. (*Note.* In adult bones the patterns are only discernible in radiographs of *isolated* specimens and would not be revealed by tomography.)

Testut (1889) describes the conditions of sternal fissure and sternal foramen and then states; 'Un degré moins avancé de l'anomalie précédente est la réunion, sur la ligne médiane, de deux ou plusieurs pièces homologues par des synchondroses verticales.' My findings not only confirm this opinion but lead me to the following conclusions. The minority of human sterna are narrow, and the relatively greater width of the majority of human sterna is fundamentally an expression of bilateral ossification. Thus, in the 'normal' human sternum the greater width of the lower part of the mesosternum is causally related to the defects mentioned above. We have four conditions of the sternum which may be arranged in sequence as follows: (i) *fissura sterni*, (ii) sternal foramen, (iii) manifest bilateral ossification, (iv) relative wideness (types II and III).

All these conditions are aetiologically connected. The difference between them is merely a difference in the degree of co-ordination which existed, during the first 3 months of embryonic life, between the forces tending to cause fusion of the lateral sternal bands and the forces tending to cause persisting separation of these bands. (These opposing forces will be discussed in a subsequent paper.)

Superimposed on the state of equilibrium achieved between these 'opposing forces' will be the variability in (a) the time of appearance, and (b) the osteogenic activity, of the individual ossification centres. These in turn must vary with such local factors as vascular pattern and such general factors as hormonal activity and food supply.

SUMMARY AND CONCLUSIONS

It is not claimed that the method of 'typing' sterna described in this communication is so exact as to be statistically any more than a useful guiding principle. It is claimed, however, that the following assertions may justifiably be made.

(i) Complete cohesion of the lateral sternal bands during embryonic stages predisposes to the development of a series of single mid-line centres of ossification during the foetal stage, and to the production of a definitive mesosternum which is narrow and has parallel sides, that is a sternum of type I.

(ii) Incomplete cohesion of the lateral sternal bands, indicated by longitudinal grooving in early foetal stages, predisposes to bilateral ossification and to the production of a sternum of type II or type III according to whether the grooving (incomplete fusion) affects only the lower half or the whole length of the mesosternum respectively.

(iii) Still less intimate union of the sternal bands would lead to the formation of a sternal foramen.

(iv) Partial or complete non-union would lead to partial or complete sternal fissure.

(v) For ease of classification it may be said that, generally, any one of three basic patterns of ossification may be found in the mesosternum, and each pattern leads to a characteristic variation in the shape of the definitive bone.

(vi) Numerous minor variations from these three basic forms occur, but each of such variants is only infrequently encountered. Nevertheless, these intermediate forms serve to link together the three basic forms, so that in a large series of specimens one may find a complete gradation from the narrow sternum through sterna which are wide below, wide in the mid-portion, wide above, or wide throughout their length; to sterna which are fenestrated; thence to sterna which are fissured below, in the middle, above or throughout their length; and finally to such conditions as *ectopia cordis thoracica*.

(vii) Neither *sex* nor *race* seem to play any very significant part in the production of any particular type of sternum.

I am indebted to Prof. G. A. G. Mitchell, Manchester University, in whose department this work has been done, and who has given me much valuable advice; to Prof. A. Galloway, Makerere College, Uganda, and Prof. R. G. Harrison, Liverpool University, for permission to study collections of material; to numerous pathologists in the Manchester area who have kindly helped to build up my own collection of specimens; and to Messrs G. Wild (Uganda), J. G. Graham and P. Howarth (Manchester) for the radiography and photography.

REFERENCES

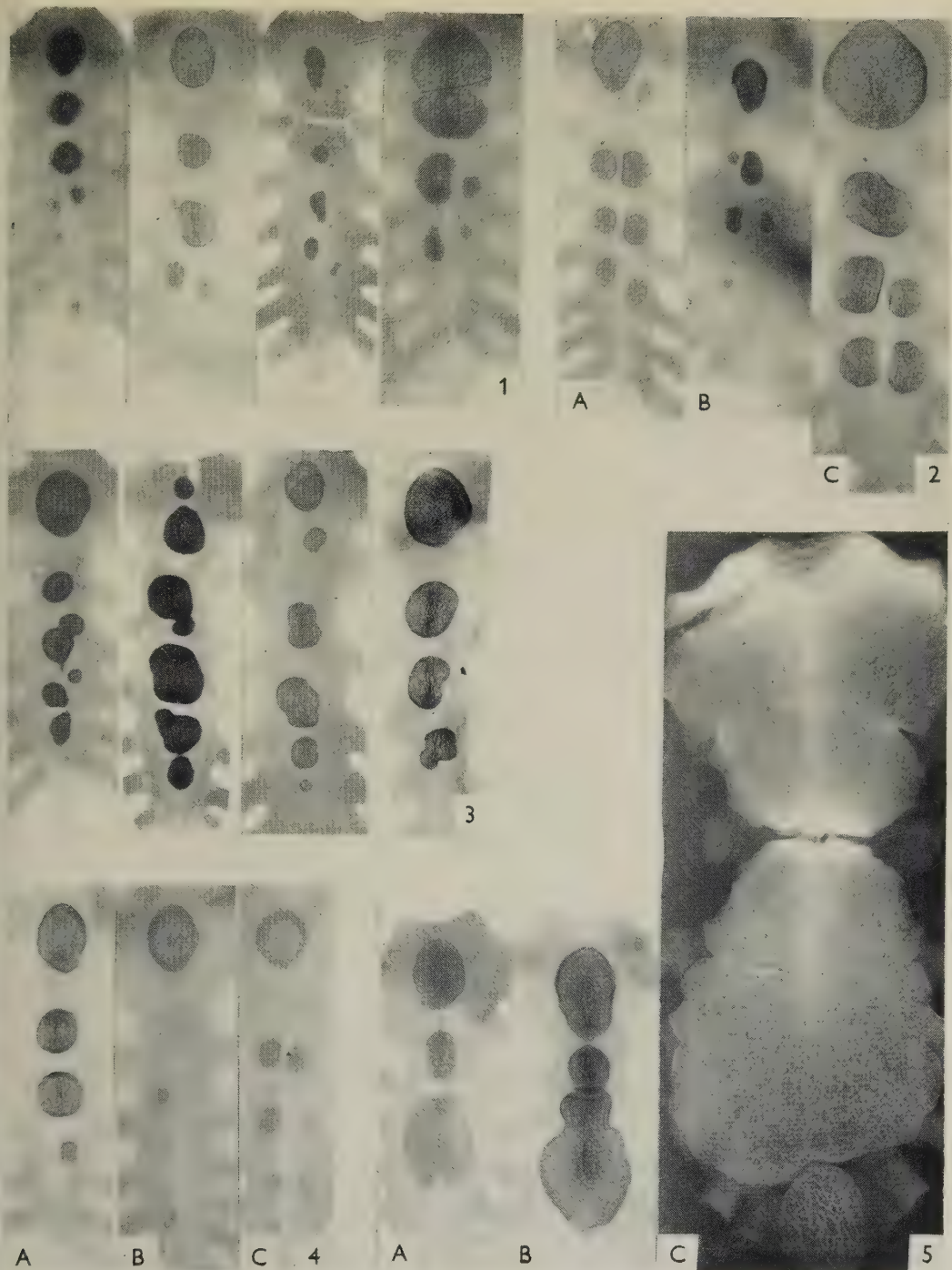
- ASHLEY, G. T. (1951). Observations on the human sternum. *J. Anat., Lond.*, **85**, 412.
ASHLEY, G. T. (1953). Typing of the human sternum—an analysis of the ossification pattern in 520 sterna in the developmental stages. *J. Anat., Lond.*, **87**, 439.
ASHLEY, G. T. (1955). The influence of sex and age on the measurements of the human sternum. *J. Forensic Med.* (in the Press).
BARTHOLIN, C. (1668). *Anatomy*, p. 353. Trans. by N. Culpeper and A. Cole. London.
BLUMENBACH, J. (1786). *Geschichte und Beschreibung der Knochen des menschlichen Körpers*. Göttingen. Quoted by Bogusat (1902).
BOGUSAT, H. (1902). 'Anomalien und Varietäten des Brustbeins', pp. 39. Inaug. Diss. Med. Königsberg.
BROC, P. P. (1836). *Traité Complet d'anatomie*, vol. 3, p. 20. Paris.
CLOQUET, H. (1828). *A System of Human Anatomy*, pp. 26–28. (Translated from the 4th edn. of the French by Robert Knox, M.D., F.R.S.E.) Edinburgh.
COITER, V. (1573). *Externarum et internarum principalium humani corporis partium tabulae, etc.*, Cap 8—De Sterno. Noribergae, pp. 60–61.
CROOKE, H. (1615). *A Description of the Body of Man, etc.* p. 743. London.
DEBIERRE, C. (1890). *Traité élémentaire d'anatomie de l'homme*. Paris: F. Alkon.
DIEMERBROECK, ISBRANDUS DE (1679). *Anatome Corporis Humani*, p. 816. Geneva.
DIEMERBROECK, ISBRANDUS DE (1685). *Opera omnia anatomica et medica*, p. 549. Ultrajecti.
EUSTACHIUS, B. (1520–74). *Opuscula Anatomica*, 2nd edn. (Accedit Leal Lealis. Lvdg. Bat. 1707.)
EUSTACHIUS, B. (1520–74). *Tabulae Anatomicae*. Rome, 1714.
FÖRSTER, A. (1865). *Die Missbildungen des Menschen*, p. 104. Jena.

- FREY, H. (1935). Ueber die Form des menschlichen Brustbeins. *Morph. Jb.* **76**, 516–569.
- GIBSON, G. A. & MALET, H. (1879). Presternal fissure, uncovering the base of the heart. *J. Anat., Lond.*, **14**, 1–9.
- GRANT, J. C. B. (1944). *A Method of Anatomy*, 3rd edn. p. 477. Baltimore.
- GRAY, H. (1946). *Anatomy*, 29th English edn. p. 98. London: Longmans Green and Co. (Edited by T. B. Johnston and J. Whillis.)
- GREIG, D. M. (1926). Cleft Sternum and Ectopia Cordis. *Edinb. Med. J.* **33**, 480–511.
- HERDNER (1947). Le sternum de l'enfant. Fréquence actuelle de troubles de développement révélés par l'examen radiographique. *J. Radiol. Électrol.* **28**, 387–393.
- KEITH, SIR A. (1948). *Human Embryology and Morphology*, 6th edn. p. 586. London: E. Arnold and Co.
- KERCKRING, T. (1685). In *Bibliothecae Anatomicae*, Vol. 2, pt. 4, Cap 15, Osteogenia Foetuum, p. 519. Geneva: J. A. Chovët.
- LICKLEY, J. D. (1904). On the morphology and development of the human sternum. Thesis presented for degree of M.D., Glasgow University.
- LUBOSCH, W. (1920). Formverschiedenheiten am Körper des menschlichen Brustbeins und ihr morphologischer und konstitutioneller Wert. *Morph. Jb.* **51**, 91–140.
- LUSCHKA, H. (1863). *Die Anatomie des Menschen. Anatomie der Brust*, pp. 86–92. Tübingen.
- MALGAIGNE, J. F. (1859). *Traité d'anatomie chirurgicale et de chirurgie expérimentale*, pt. 2, p. 101. Paris.
- MARKOWSKI, J. (1902). Ueber die Varietäten der Ossification des menschlichen Brustbeins u. über deren morphologische Bedeutung. *Poln. Arch. biol. med. Wiss.* **1**. Quoted by Markowski (1905).
- MARKOWSKI, J. (1905). Sollte der Verknöcherungsprozess des Brustbeins von keiner morphologischen Bedeutung sein. *Anat. Anz.* **26**, 248–269.
- MARTIN, R. (1914). *Lehrbuch der Anthropologie*, p. 902. Jena.
- MASSA, N. Quoted by Riolanus, J. (1649).
- MERKEL, FR. (1899). *Handbuch der topographischen Anatomie*, Bd. II, pp. 314–319. Braunschweig.
- MONRO, A. (1758). *The Anatomy of the Human Bones and Nerves*, 6th edn. p. 222. Edinburgh.
- PANSCH, A. (1875). Über Anomalien am Thoraxskelet. *Reich u. du Bois. Reym. Arch.* Quoted by Bogusat (1902).
- PATERSON, A. M. (1904). *The Human Sternum*, pp. 89. London.
- RATHKE (1838). Zur Entwicklungsgeschichte der Thiere, eine Bemerkung. *Müller's Arch. Jahrg.* p. 361.
- RHEAD, ALEXANDER (1634). *A Description of the Body of Man*, p. 4. London: T. Cotes.
- RIOLANUS, J. (1649). *Encheiridium Anatomicum et Pathologicum*, Lib. VI, cap. XIV. Ludg. Batav, p. 442.
- RUGE, G. (1880). Untersuchungen über Entwicklungsvorgänge am Brustbein und an der Sternoclavicularverbindung des Menschen. *Morph. Jb.* **6**, 362–414.
- SCHULTZ, A. H. (1944). Age changes and variability in Gibbons. A morphological study on a population sample of a man-like ape. *Amer. J. Phys. Anthropol.* (N.S.), **2**, 1–129.
- STIEVE, H. & HINTZSCHE, E. (1925). Ueber die Form des menschlichen Brustbeins. *Z. Morph. Anthr.* (1923–25), **23**, 361–410.
- TARIN, M. (1753). *Ostéographie ou description des os de l'adulte du foetus, etc.*, pp. 94–95. Paris.
- TESTUT, L. (1889). *Traité d'anatomie humaine*, 1st edn., vol. I, p. 82. Paris.

EXPLANATION OF PLATES

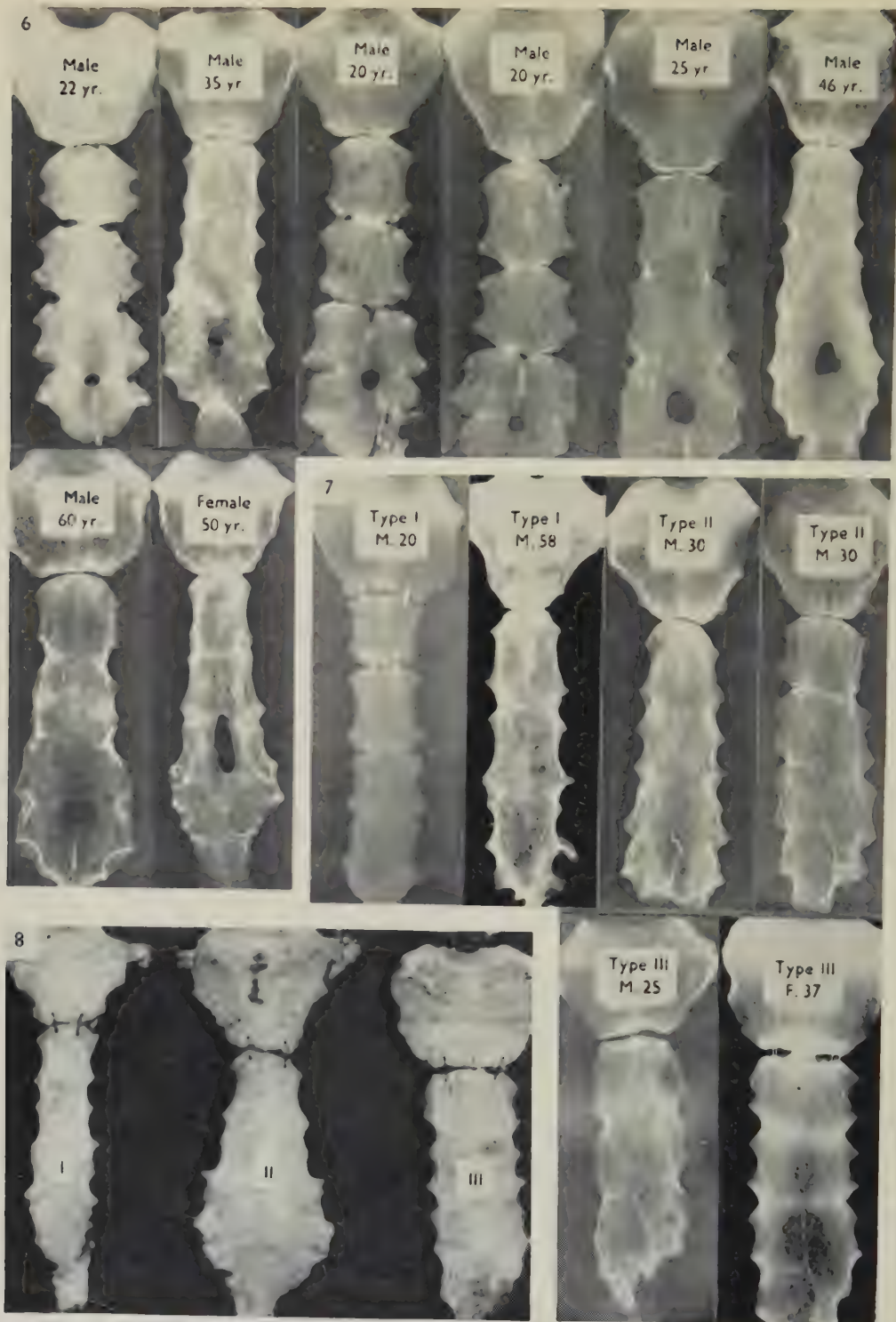
PLATE I

- Fig. 1. In the examples shown, one centre of each 'pair' of centres has apparently appeared later than its fellow.
- Fig. 2. Selected specimens to show how the ossification pattern may appear to change from type III to apparent type II as development proceeds.
- Fig. 3. Specimens which suggest that the ossification pattern may appear to change to type I from type II.



ASHLEY—PATTERN OF OSSIFICATION AND DEFINITIVE SHAPE OF MESOSTERNUM IN MAN

(Facing p. 104)



ASHLEY—PATTERN OF OSSIFICATION AND DEFINITIVE SHAPE OF MESOSTERNUM IN MAN

Pattern of ossification and definitive shape of mesosternum in man 105

Fig. 4. Radiographic evidence of 'grooving' of foetal sterna. (The presence of grooving was confirmed by inspection.)

Fig. 5. Indicating (C) an unusual form of mesosternum, and (A, B) the type of precocious ossification which may have led to its formation.

PLATE 2

Fig. 6. Illustrating the association of mesosternal foramen with the type II pattern of ossification. Represented here are eight of the thirteen African sterna presenting this condition.

Fig. 7. Radiographs of selected specimens of adult sterna revealing patterns of ossification typical of types I-III.

Fig. 8. Photographs to show the distinctive shapes of the three types of sternum.

TRANSITIONAL EPITHELIUM AND OSTEOGENESIS

BY F. R. JOHNSON AND R. M. H. McMINN

Department of Anatomy, University of Sheffield

INTRODUCTION

Heterotopic bone formation has been noted by several observers (e.g. Neuhof, 1917) in the connective tissue surrounding the urinary bladder following operations on this viscus. Huggins (1931) was the first to demonstrate the association of such bone formation with bladder mucosa. As a result of his investigations on a large number of dogs, he suggested that transitional epithelium is capable of inducing osteogenesis in certain types of connective tissue. Subsequent workers have confirmed his observations on dogs (Regen & Wilkins, 1934; Huggins, McCarroll & Blocksom, 1936; Gomori, 1943; Abbott & Stephenson, 1945; Marshall & Spellman, 1954; Boyarsky & Duque, 1955), and the phenomenon has also been found to occur in rats (Huggins *et al.* 1936), guinea-pigs (Huggins *et al.* 1936; Gomori, 1943; Loewi, 1954) and rabbits (Huggins, 1931; Gomori, 1943). The epithelium of the gall bladder also appears to be capable of exerting an osteogenic stimulus (Huggins & Sammett, 1933).

Many other workers have investigated experimentally the formation of bone by induction. Urist & McLean (1952) tested various tissues for their inducing influence when placed in the anterior chamber of the eye. Several investigators, quoted by Levander (1949), have claimed that extracts of certain bone elements are capable of inducing bone formation in rabbit muscle, while others have shown that similar results can be obtained by the use of non-specific irritants (see Hartley & Tanz, 1951). Attempts have been made to influence the healing of fractures by placing bladder epithelium in their vicinity (Copher, Key & West, 1932) or by the local injection of extracts of bladder mucosa (Eskelund & Plum, 1950).

In the study of heterotopic bone formation the inducing agent and the induced tissue must be considered. Hitherto, the nature of the inducing agent remains obscure, and in most of the investigations mentioned, comparatively little attention has been paid to the early reactions in the tissues in which bone formation has taken place. Accordingly, in the present experiments on the cat, attention has been specially focused on the connective tissues in which osteogenesis is occurring. Since previous work on the osteogenesis induced by bladder epithelium has been confined almost exclusively to autogenous material, the possibility of bone formation following the use of homogenous implants has also been investigated. At the same time, histochemical observations have been made on bladder epithelium in its normal site and when implanted.

MATERIAL AND METHODS

The operations on healthy adult cats were similar to those which the authors have described previously, during the study of the behaviour of implantation grafts of bladder mucosa (Johnson & McMinn, 1955*a*). From each animal, under nembutal

anaesthesia, about 1 cm.² of bladder mucosa was removed and in most cases divided into four pieces. One of these was immediately fixed in ice-cold 80 % alcohol and another in ice-cold Carnoy's fluid for histological study. The remaining two pieces were used as implantation grafts, one being implanted into the sheath of the rectus abdominis muscle of the same cat, the other into the rectus sheath of another cat.

In order to determine whether the reactions surrounding the implantations were the result of a specific influence of bladder mucosa, autogenous implants of adipose tissue from the anterior abdominal wall were inserted into the sheath of the rectus abdominis muscle in a number of animals, and the results were studied by the methods used for the mucosal implants.

A total of forty-three animals was used, and since most of these animals received both an autograft and a homograft of bladder mucosa, thirty-seven autografts and thirty-nine homografts were available for study.

The animals were killed 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18, 28 or 48 days after operation; in most cases three autografts and three homografts were thus obtained for each of the above periods.

Histological technique

The implantation site was removed and divided into 2. One portion was fixed in ice-cold 80 % alcohol for 18 hr., the other in ice-cold Carnoy's fluid for a similar period. When dividing the implantation site into 2 the presence of calcified bone was detected by the grating of the knife, in which case the tissues were decalcified by immersion in a citrate buffer (Gomori, 1952) so that the presence of calcium would not interfere with the interpretation of other histological and histochemical findings. These tissues and those fixed at the time of operation were embedded and serially sectioned at 8 μ . Every 20th section was mounted and stained with haematoxylin and eosin. Serial sections in regions of particular interest were mounted for histochemical study.

For the demonstration of alkaline phosphatase the Gomori technique was employed on the alcohol-fixed material, using sodium- β -glycerophosphate as substrate. Incubation periods ranging from 5 min. to 24 hr. were used. Controls were performed by omitting the sodium- β -glycerophosphate from the incubating fluid.

A number of staining methods was used for the demonstration of glycogen on material fixed in Carnoy's fluid or in alcohol. Best's carmine method, and the silver technique advocated by Pritchard (1949), proved to be less satisfactory than the periodic acid-Schiff (P.A.S.) method of Hotchkiss (1948), and throughout most of the investigation this latter technique alone was used. Adjacent sections treated with saliva served as controls.

Cytoplasmic basophilia and metachromasia were studied by several methods on material fixed in Carnoy's fluid. Although some sections were stained with thionin, alcian blue or methyl green pyronin, toluidine blue (0.2 % solution for 30 min.) was used in most cases. Control sections were stained after treating overnight with 10 % perchloric acid at 4° C. (Seigel & Worley, 1951).

RESULTS

General observations

Transitional epithelium invariably formed a cyst by migration and growth of epithelial cells from the margins of the graft. In the early stages, the cells which had grown and spread from the margins (hereafter referred to as spread epithelium) showed different characteristics from those of the graft itself (graft roof epithelium), in that the cells of the spread epithelium were larger (cf. Pl. 2, figs. 19, 20), the cytoplasm stained less densely with haematoxylin and eosin, and the nuclei had a more vesicular appearance. Later the cells reverted to normal. Increased mitotic activity was present in the epithelium from the 2nd to the 8th days, both in the spread and in the graft roof epithelium.

These findings were similar up to the 8th day in both autografts and homografts.

In addition to the epithelial mitosis, there was a striking increase in the activity of the connective tissue surrounding the cyst. This became evident on the 4th day and could be recognized by the presence of numerous loosely packed fibroblasts whose large, irregular, cytoplasmic processes were easily seen. Many of these fibroblasts were undergoing mitosis (Pl. 1, figs. 1-3). This activity persisted until approximately the 10th post-operative day, after which mitotic figures were more rarely seen, and although the fibroblasts had become more closely packed they could still be recognized as young cells. The activity occurred around the whole cyst and seemed to be as active in relation to the base of the graft itself as it was to the spread epithelium.

Similar appearances in the surrounding connective tissue were recognized following the implantation of a portion of the animal's own adipose tissue (Pl. 1, fig. 4). A fact of possible significance was the lack of activity in the fibrous tissue of the graft compared with that seen in the implantation site (Pl. 1, fig. 5).

During the 2nd week the homografts became infiltrated with lymphocytes and plasma cells. At first the subepithelial tissues alone were involved, but later there was invasion of the epithelium, which was destroyed in the 3rd or 4th week (Johnson & McMinn, 1955*a*). The site of the cyst was eventually occupied by organizing granulation tissue. The epithelium of autografts persisted in a healthy state at all stages examined.

Bone formation

In this investigation bone formation occurred following the implantation of both autografts and homografts. The earliest time at which bone tissue was detected was the 10th day. Twenty-three animals were allowed to survive for 10 days or longer, and of these sixteen received both an autograft and a homograft, three an autograft alone and four a homograft alone. Of the sixteen animals which received both grafts, bone was found associated with both types of graft in seven, with the autograft alone in five, and with the homograft alone in one. Three of the animals showed no evidence of osteogenesis. Of the three animals which received autografts alone, bone was found in all, and of the four which received homografts alone, bone was found in two. Hence in this series of twenty-three animals, bone was found in fifteen out of nineteen autografts and in ten out of twenty homografts.

Bone formation always occurred deep to the spread epithelium; on no occasion was it found in relationship to the graft roof epithelium. The bone first appeared among the surrounding connective tissue, which, as shown above, consisted of young actively mitotic cells. Usually the bone was not in direct contact with the epithelium but was separated from it by a thin band of connective tissue (Pl. 1, fig. 6).

In homografts, bone formation occurred prior to the time of destruction of the epithelium, and as in autografts was always found underlying the spread epithelium (Pl. 2, fig. 18). In specimens which were examined after epithelial destruction had taken place, the bony plaque appeared to be surrounded by organizing connective tissue.

Before the onset of bone formation, no cells resembling cartilage were found. In the formation of the bone, the appearances closely resembled those normally seen in intramembranous ossification (e.g. Bevelander & Johnson, 1950; Pritchard, 1952). Different stages in this process are illustrated in Pl. 1, figs. 9, 7 and 6.

Although no exact measurements were made, the size of the bony plaque seemed to approximate to that of the implant. There did not appear to be any increase in size after the 3rd week. This latter contention is supported by the fact that periosteum and endosteum became less cellular about that time, and that the sizes of all plaques examined at 21 days and later were approximately the same.

Histochemical observations

Alkaline phosphatase

Although it is well recognized that histochemical techniques for the demonstration of alkaline phosphatase are not reliable as a means of determining the absolute quantities of this enzyme in any tissue, the authors have been impressed by the extreme variability of the results in the epithelium of different urinary bladders when a carefully standardized Gomori technique is used. In some animals the epithelium gave a strong reaction (Pl. 1, fig. 8), while in others the distribution was patchy even after prolonged incubation periods (Pl. 1, fig. 10). Apart from the subepithelial capillary plexus the tissues deep to the epithelium of the bladder were invariably negative.

When portions of bladder mucosa, adjacent to those which have just been described, were transplanted and studied at later dates, using precisely the same technique, it was found that the reaction of the graft roof epithelium in autografts varied little from that seen at the time of operation; occasionally there appeared to be an increase, but sometimes there was a decrease in the intensity of the reaction. The spread epithelium also showed some variability. The reactions of the subepithelial tissues differed, depending on their relationship to the overlying epithelium. The tissues deep to the graft roof epithelium remained negative, while that underlying the spread epithelium and the graft itself gave, from the 3rd to the 10th days, an extremely dense reaction (Pl. 2, fig. 11). The tissue which gave the reaction was that which had been noted above to show increased mitotic activity. After the 10th day, the reaction in this tissue gradually decreased, and in the later stages which have been studied it was completely negative. The appearances with homografts were similar.

When osseous tissue was first recognized, the osteoblasts and the intercellular substance in the region of ossification gave a strong reaction. Later, when further cellular elements were definable, it was found that the osteoblasts and fibroblasts of the periosteum, as well as the osteocytes and intercellular substance, were positive (Pl. 2, fig. 14). Once bone canals were recognizable the endosteum was also positive (Pl. 2, fig. 12). In subsequent stages the matrix first became negative to be followed rapidly by the osteocytes (Pl. 2, fig. 12). Later, when the periosteum no longer showed evidence of cellular activity, it also gave a negative reaction. The endosteal cells, however, remained positive at all periods examined (Pl. 2, fig. 13).

Glycogen

Unlike the variable results for alkaline phosphatase, the findings for glycogen (with the P.A.S. technique) showed a remarkable constancy. Bladder epithelium removed at the time of operation gave a strong reaction (Pl. 2, fig. 15) in almost all the animals used. The glycogen was confined mainly to the superficial and middle layers of cells and seldom was any seen in cells of the basal layer.

The intensity of the reaction in epithelium that had been implanted into the rectus sheath for at least 3 days was considerably less than that in an adjacent piece of epithelium removed from the same bladder at the time of operation (Pl. 2, fig. 17). Not only was the intensity reduced but the distribution was altered, in that glycogen was evenly distributed throughout all layers of the epithelium.

Glycogen was present in the spread epithelium in a concentration comparable with that found in the graft roof. These findings were similar in both autograft and homograft epithelium. The implanted epithelium did not regain its former concentration of glycogen during the periods studied; in autografts the appearances noted at the 3rd and 4th days remained similar up to the 48th day.

In spite of the fact that careful search was made for the presence of glycogen in the osteoblasts, the authors were unable to convince themselves of its presence. This may have been due to the insensitivity of the P.A.S. technique, though it may be noted that Schajowicz & Cabrini (1954) failed to demonstrate it in some instances during intramembranous ossification.

Metachromasia and cytoplasmic basophilia

Metachromasia was an obvious feature in the tissues surrounding both autografts and homografts following the staining of the tissues with basic dyes. It became apparent on the 2nd day, increased during the 1st week and thereafter gradually decreased. The metachromasia occurred in the connective tissue that showed the increase in mitotic activity and the presence of alkaline phosphatase, i.e. tissues underlying both the graft and the spread epithelium. It was never found in the tissues of the graft itself.

Normal bladder epithelium showed a constant degree of cytoplasmic basophilia which was especially prominent in the deeper layers of cells (Pl. 2, fig. 16). As soon as there was evidence of increased mitosis in the epithelium following implantation, there was a pronounced increase in the basophilia (Pl. 2, fig. 19), which later reverted to a normal level when mitotic activity diminished. In the early stages, the spread

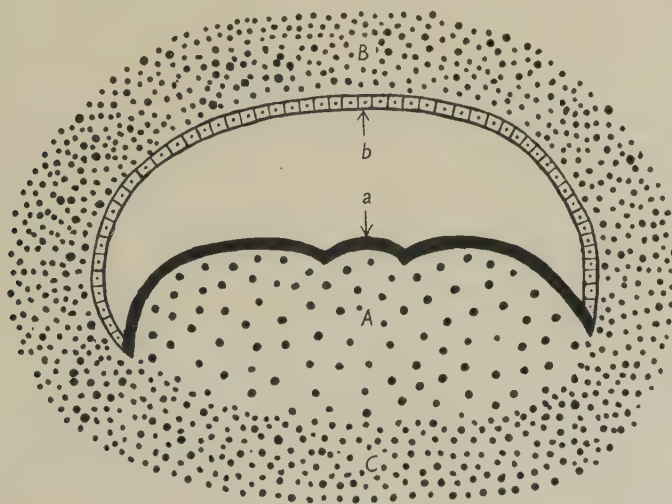
epithelium showed a decrease in basophilia, which may have been a dilution effect due to the large size of the cells (Pl. 2, fig. 20). The findings were similar in both autografts and homografts up to the time of destruction of the homograft epithelium.

During the period when the fibroblasts showed marked mitotic activity they also showed increased basophilia. This increase was a most striking feature when these active cells were compared with the inactive fibroblasts of the subepithelial tissues of the graft (Pl. 1, fig. 5). As with the phosphatase reaction, the basophilia decreased shortly after these cells showed a lowered mitotic rate.

The basophilia of the cells at the site of ossification was similar to that described in histochemical studies of intramembranous ossification (e.g. Pritchard, 1952). The cytoplasm of the large osteoblasts with eccentric nuclei stained deeply, while that of the osteocytes was pale.

DISCUSSION

The results of the present experiments demonstrate that, as in other animals, bone is formed in the rectus sheath of the cat following implantation of bladder mucosa. It has also been shown that homografts are as successful as autografts in the production of bone.



Text-fig. 1. A diagrammatic representation of an implantation cyst of bladder mucosa. *A*, subepithelial tissue of the graft; *a*, graft roof epithelium; *B*, subepithelial tissues underlying spread epithelium *b*; *C*, connective tissue deep to graft.

When bladder mucosa is implanted into the rectus sheath, a cyst is formed (see Text-fig. 1). This cyst is lined by the epithelium (*a*) of the graft (*A*), and by epithelium (*b*) which has spread and grown from the margins of the graft. As it is impossible to separate the epithelium of the graft from the subepithelial tissues at the time of implantation, it will be realized that the subepithelial tissues of the graft are part of the urinary bladder, whilst the connective tissue (*B*) which underlies the

spread epithelium, and that (C) underlying the graft itself has arisen from the fibrous tissue of the rectus sheath. The reactions of these tissues are markedly different: that which is basically part of the graft remains quiescent, while that which underlies both the spread epithelium and the graft itself shows several features of interest. First, between the 4th and 9th days, proliferative activity is evident, as indicated by numerous mitotic figures and young fibroblasts. Secondly, it is in these same regions and at the same time that a positive reaction for alkaline phosphatase, metachromasia and increased basophilia are present. That these reactions are not the result of a specific influence of the implanted mucosa is shown by the fact that similar responses are seen in the connective tissue surrounding the implants of adipose tissue. It would appear, therefore, that these reactions are invoked by the presence of a 'foreign body'.

It has been noted that bone, when present, lies in the tissues underlying the spread epithelium (B in Text-fig. 1), confirming the findings of Huggins (1931) and others. The origin of osteoblasts in abnormal sites has given rise to considerable speculation. That fibroblasts can undergo metaplasia and form osteoblasts has been upheld by several workers, e.g. Huggins (1937) and Maximow & Bloom (1952). In the present work it has been seen that both fibroblasts and osteoblasts give a positive reaction for alkaline phosphatase and show marked cytoplasmic basophilia, findings which tend to indicate that the metabolism of these two types of cell may at this stage be similar. Bradfield (1950) states that an important cellular activity frequently associated with the presence of phosphatases is the metabolism of nucleic acids and proteins, especially fibrillar proteins. The presence of cytoplasmic basophilia in the cells under discussion supports this contention, since Caspersson (1950) and Brachet (1950) have shown that basophilia is indicative of nucleic acid formation. The contention that phosphatase is concerned with the elaboration of protein is well supported. Fell & Danielli (1943) showed that collagen formation in healing wounds is associated with an increase in the phosphatase level in the fibroblasts themselves. They also demonstrated that in vitamin C deficiency there is a close correlation between the degree of vitamin C deficiency, the rate of collagen formation and the level of alkaline phosphatase. Similar observations have been made by Moog (1946) on the relationship of phosphatase to the formation of an osteoid matrix in normal osteogenesis, and by Bourne (1943) who showed that in scorbutic animals both enzyme and osseous matrix are scarce during the healing of bone wounds. It would thus seem that fibroblasts and osteoblasts possess metabolic equipment of such a nature that a transition from one type to the other would seem feasible.

On the other hand, some authorities (e.g. Ham, 1953) consider that fibroblasts are fairly highly differentiated cells, and so unlikely to change their type. Where osteoblasts arise *de novo*, it is suggested that they are formed under the influence of a changed environment from poorly differentiated mesenchymal cells. Applying this concept to the present experiments, it may be that the poorly differentiated cells that exist under both the graft roof and the spread epithelium show different responses to the inducing influence, due to the fact that under one type of epithelium—the spread—a 'foreign body' reaction has been set up in their neighbourhood. In this case the mesenchymal cells near rapidly dividing fibroblasts may be

capable of differentiating into osteoblasts, while those in the subepithelial tissues of the graft remain quiescent. This argument presupposes that the inducing agent, whatever it may be, is acting equally upon the mesenchymal cells underlying both types of epithelia.

As to the origin of the inducer which causes the formation of osteoblasts, there seems to be little doubt. Huggins (1931) tested the efficacy of the various components of the bladder graft, and came to the conclusion that the epithelial element was responsible, a contention that has been supported by subsequent workers. The influence of the epithelium upon the surrounding cells may be produced by the liberation of some chemical substance which diffuses into the subepithelial tissues. Since bone is not found in the connective tissue of the graft itself (*A* in Text-fig. 1) it may be because diffusion of the chemical substance does not readily take place in this latter tissue, and this may also explain why the tissues (*C*) deep to the graft, which show the same reaction as those (*B*) deep to the spread epithelium, never show evidence of bone formation.

The possibility must be considered that, during the formation of heterotopic bone, alkaline phosphatase is the inducing agent. Blum (1944) found that when pellets containing alkaline phosphatase were implanted into rabbit muscle, bone was formed. However, Slessor & Wyburn (1948), who carried out a similar procedure, were unable to confirm his results, and Johnson & McMinn (1955*b*) were unsuccessful in inducing the formation of heterotopic bone in the rectus sheath following the implantation of intestinal mucosa which is known to be one of the richest sources of this enzyme. The fact that bone was not always formed in the current experiments despite the presence of phosphatase in the epithelium suggests that in addition to or apart from phosphatase other factors may be involved.

The function of glycogen in cells such as those of liver and skeletal muscle is well understood, but the reason for its presence in various epithelia, including that under discussion, is obscure. One theory to explain the presence of glycogen in the superficial cells of epithelia has been advanced by Dempsey & Wislocki (1944) and supported by the investigations of Bradfield (1951). These workers maintain that the accumulation of glycogen is an adaptation feature brought about by a reduction in oxygen tension. While lowered oxygen tension in the superficial cells of epithelia consisting of many layers can be readily appreciated, this concept is more difficult to apply to transitional epithelium which is normally relatively thin and which has a well-defined subepithelial capillary plexus of blood vessels. If glycogen does accumulate as a result of a lowered oxygen tension, it would seem highly probable, when it is remembered that the graft is temporarily cut off from its blood supply, that the concentration of glycogen in the cells of the graft should increase rather than decrease.

In bladder epithelium removed at the time of operation, ribonucleic acid is concentrated in the cytoplasm of the lower layers of epithelial cells, in contrast to the glycogen which is found more superficially. During the period of mitotic activity among the epithelial cells, the basophilia of both the graft roof and the spread epithelium is increased, results which are in keeping with the high concentration of ribonucleic acid which has been shown to occur in proliferating cells and in those involved in protein synthesis (Caspersson, 1950).

It is of considerable interest that bone has been found associated with homogenous as well as autogenous implants. Bone formation in association with homografts has always been initiated prior to the onset of destruction of the epithelium. It is generally accepted that immune reactions become established within 6-7 days (e.g. Ehrlich & Harris, 1942). If it can be assumed that the immune response in the present experiments conforms to the usual pattern, then it will be seen that this reaction is established before bone first appears. Thus it might be assumed that the immune reaction does not interfere with the liberation of the inducer or with the response of the host tissues. Similarly, the presence of glycogen and ribonucleic acid in the homograft epithelium up to the time of its destruction, may indicate that there is no significant disturbance of carbohydrate or protein synthesis (cf. Scothorne & Tough, 1952; Scothorne & Scothorne, 1953). It is also of interest to note that the newly formed bone does not appear to be involved in the immunity reaction.

SUMMARY

1. Heterotopic bone formation has been studied in the cat following the implantation of bladder mucosa into the sheath of the rectus abdominis muscle.
2. Homografts are as successful as autografts in the induction of bone.
3. Bone formation occurs in connective tissue deep to epithelium which has spread from the margins of the graft. This connective tissue shows marked mitotic activity, metachromasia, cytoplasmic basophilia, and an intense reaction for alkaline phosphatase.
4. Transitional epithelium gives a reaction of varying intensity for alkaline phosphatase. Constant levels of glycogen and cytoplasmic basophilia are found.
5. Possible factors involved in bone induction are discussed.

We are indebted to Prof. Francis Davies for his helpful criticism in the preparation of this paper. We thank Messrs J. H. Kugler, J. H. Morill and D. A. Allen for technical assistance, and Mr Kugler for the preparation of photomicrographs. Part of the expenses of this work was defrayed by a grant from the Medical Research Fund of the University of Sheffield, for which we also wish to express our thanks.

REFERENCES

- ABBOTT, A. C. & STEPHENSON, E. (1945). Further observations on experimental bone formation with special reference to the bone-forming properties of the epithelial lining of the trigone in the dog. *Canad. med. Ass. J.* **52**, 358-362.
- BEVELANDER, G. & JOHNSON, P. L. (1950). A histochemical study of the development of membrane bone. *Anat. Rec.* **108**, 1-21.
- BLUM, G. (1944). Phosphatase and the repair of fractures. *Lancet*, **2**, 75-78.
- BOURNE, G. H. (1943). Some experiments on the possible relationship between vitamin C and calcification. *J. Physiol.* **102**, 319-328.
- BOYARSKY, S. & DUQUE, O. (1955). Ureteral regeneration in dogs: an experimental study bearing on the Davis intubated ureterotomy. *J. Urol.* **73**, 53-61.
- BRACHET, J. (1950). The localisation and the role of ribonucleic acid in the cell. *Ann. N.Y. Acad. Sci.* **50**, 861-869.
- BRADFIELD, J. R. G. (1950). The localization of enzymes in cells. *Biol. Rev.* **25**, 113-157.
- BRADFIELD, J. R. G. (1951). Glycogen of vertebrate epidermis. *Nature, Lond.*, **167**, 40-41.
- CASPERSSON, T. O. (1950). *Cell Growth and Cell Function*. London: Chapman and Hall Ltd.

- COPPER, G. H., KEY, J. A. & WEST, E. S. (1932). Influence of bladder extracts and viosterol on healing of fractures and bone defects. *Proc. Soc. exp. Biol., N.Y.*, **29**, 646-648.
- DEMPSEY, E. W. & WISLOCKI, G. B. (1944). Observations on some histochemical reactions in the human placenta, with special reference to the significance of the lipoids, glycogen and iron. *Endocrinology*, **35**, 409-429.
- EHRICH, W. E. & HARRIS, T. N. (1942). The formation of antibodies in the popliteal lymph node in rabbits. *J. exp. Med.* **76**, 335-348.
- ESKELUND, V. & PLUM, C. M. (1950). Experimental investigations into the healing of fractures. *Acta orthopaed. scand.* **19**, 433-475.
- FELL, H. B. & DANIELLI, J. F. (1943). The distribution of alkaline phosphomonoesterase in experimental wounds and burns in the rat. *Brit. J. exp. Path.* **24**, 196-203.
- GOMORI, G. (1943). Calcification and phosphatase. *Amer. J. Path.* **19**, 197-209.
- GOMORI, G. (1952). *Microscopic Histochemistry. Principles and Practice*. The University of Chicago Press.
- HAM, A. W. (1953). *Histology*, 2nd ed. Philadelphia: J. B. Lippincott Co.
- HARTLEY, J. & TANZ, S. S. (1951). Experimental osteogenesis in rabbit muscle. *Arch. Surg., Chicago*, **63**, 845-851.
- HOTCHKISS, R. D. (1948). A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.* **16**, 131-141.
- HUGGINS, C. B. (1931). The formation of bone under the influence of epithelium of the urinary tract. *Arch. Surg., Chicago*, **22**, 377-408.
- HUGGINS, C. (1937). The composition of bone and the function of the bone cell. *Physiol. Rev.* **17**, 119-143.
- HUGGINS, C. B., MCCARROLL, H. R. & BLOCKSOM, B. H. (1936). Experiments on the theory of osteogenesis. The influence of local calcium deposits on ossification; the osteogenic stimulus of epithelium. *Arch. Surg., Chicago*, **32**, 915-931.
- HUGGINS, C. B. & SAMMETT, J. F. (1933). Function of the gall bladder epithelium as an osteogenic stimulus and the physiological differentiation of connective tissue. *J. exp. Med.* **58**, 393-400.
- JOHNSON, F. R. & MCINN, R. M. H. (1955a). The behaviour of implantation grafts of bladder mucosa. *J. Anat., Lond.*, **89**, 450-456.
- JOHNSON, F. R. & MCINN, R. M. H. (1955b). *Transplant. Bull.*, submitted for publication.
- LEVANDER, G. (1949). On tissue induction. *Acta path. microbiol. scand.* **26**, 113-141.
- LOEWI, G. (1954). The stimulation of osteogenesis by urinary bladder tissue. *J. Path. Bact.* **68**, 419-422.
- MARSHALL, V. F. & SPELLMAN, R. M. (1954). *Transplant. Bull.* **1**, 150-151.
- MAXIMOW, A. A. & BLOOM, W. (1952). *A Textbook of Histology*, 6th ed. Philadelphia and London: W. B. Saunders Co.
- MOOG, FLORENCE (1946). The physiological significance of the phosphomonoesterases. *Biol. Rev.* **21**, 41-59.
- NEUHOF, H. (1917). Fascia transplantation into visceral defects; an experimental and clinical study. *Surg. Gynec. Obstet.* **24**, 383-427.
- PRITCHARD, J. J. (1949). A new histochemical method for glycogen. *J. Anat., Lond.*, **83**, 30-31.
- PRITCHARD, J. J. (1952). A cytological and histochemical study of bone and cartilage formation in the rat. *J. Anat., Lond.*, **86**, 259-277.
- REGEN, E. M. & WILKINS, W. E. (1934). Phosphatase in heterotopic bone formation following transplantation of bladder mucosa. *J. Lab. clin. Med.* **20**, 250-252.
- SCHAJOWICZ, F. & CABRINI, R. L. (1954). Histochemical studies of bone in normal and pathological conditions. *J. Bone Jt. Surg.* **36B**, 474-489.
- SCOTHORNE, R. J. & SCOTHORNE, A. W. (1953). Histochemical studies on human skin autografts. *J. Anat., Lond.*, **87**, 22-29.
- SCOTHORNE, R. J. & TOUGH, J. S. (1952). Histochemical studies of human skin autografts and homografts. *Brit. J. plast. Surg.* **5**, 161-170.
- SEIGEL, G. B. & WORLEY, L. G. (1951). The effects of vitamin B12 deficiency on the cytoplasmic basophilia of rat tissues. *Anat. Rec.* **111**, 597-615.
- SLESSOR, A. & WYBURN, G. M. (1948). Phosphatase and bone formation. *Lancet*, **1**, 212-213.
- URIST, M. R. & MCLEAN, F. C. (1952). Osteogenetic potency and new-bone formation by induction in transplants to the anterior chamber of the eye. *J. Bone Jt. Surg.* **34A**, 448-470.

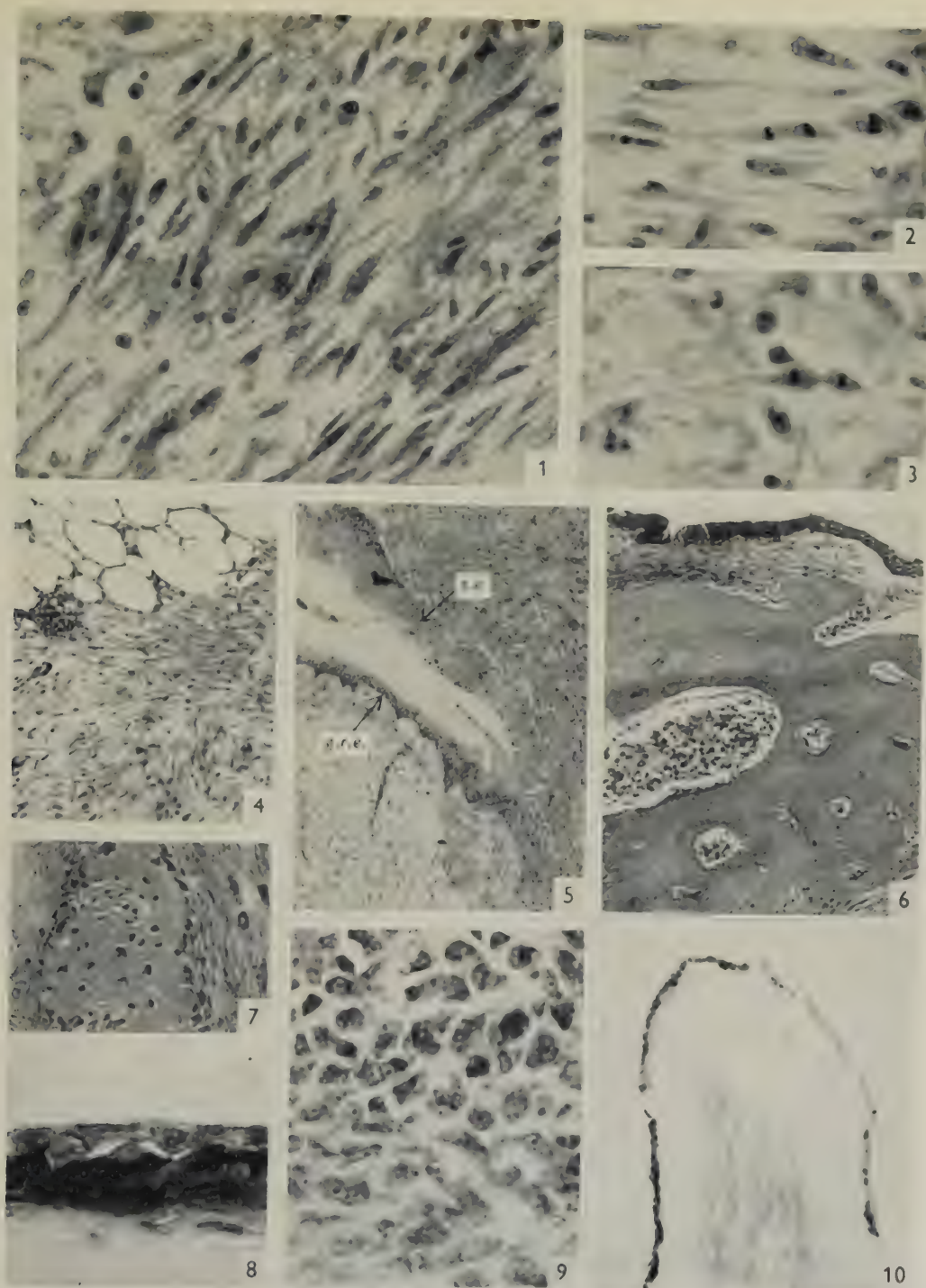
EXPLANATION OF PLATES

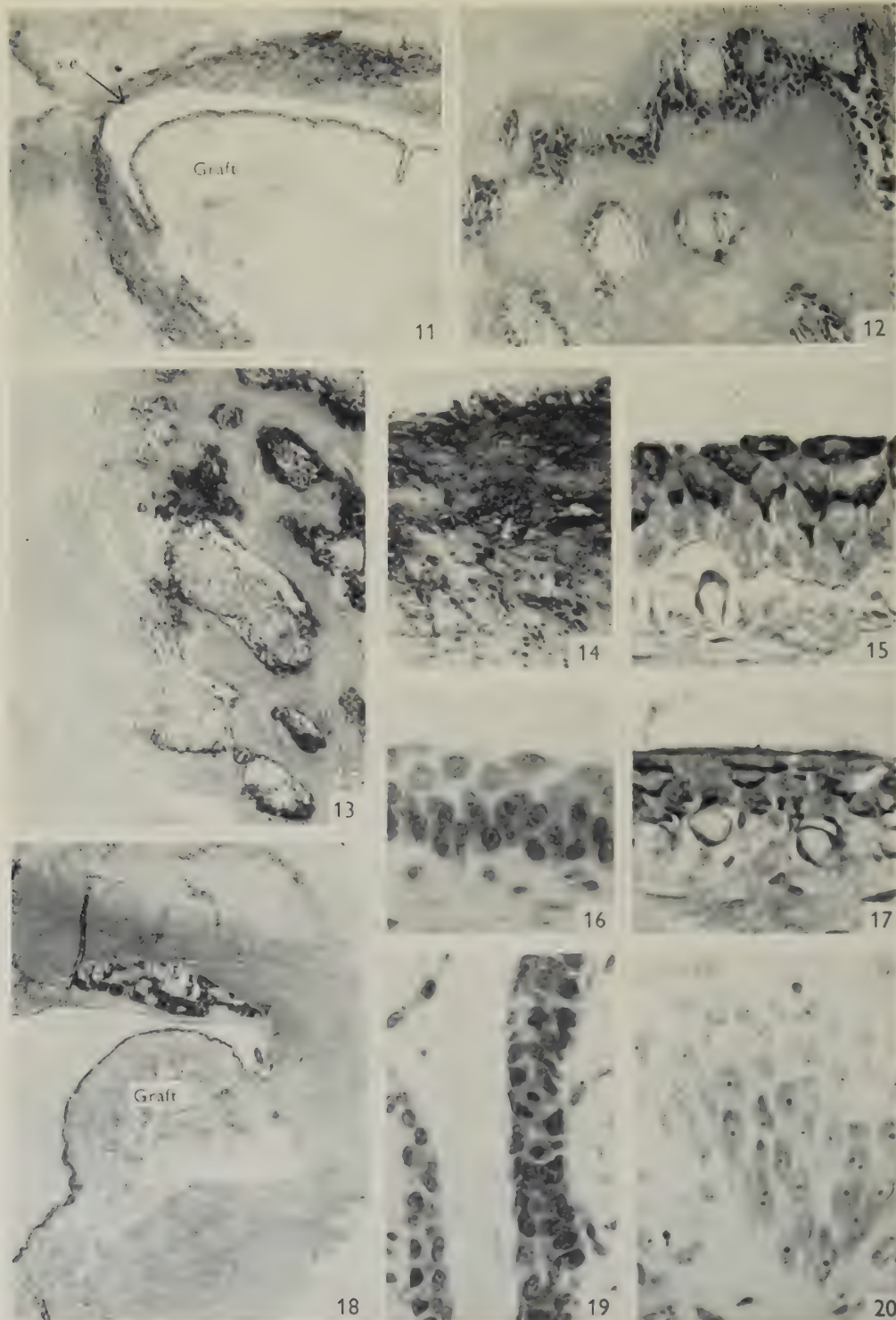
PLATE 1

- Fig. 1. Autograft. Day 5. Section demonstrating young fibroblasts with well-defined cytoplasmic processes in the tissues deep to the spread epithelium. Haematoxylin and eosin. $\times 350$.
- Fig. 2. Autograft. Day 5. Mitoses in young connective tissue. A metaphase and an anaphase are seen. Haematoxylin and eosin. $\times 550$.
- Fig. 3. Homograft. Day 6. Young fibroblast in telophase showing a well-defined cytoplasmic process. Haematoxylin and eosin. $\times 550$.
- Fig. 4. Autograft. Day 8. Section through the site of an implant of adipose tissue, showing young connective tissue surrounding the implant. Haematoxylin and eosin. $\times 88$.
- Fig. 5. Autograft. Day 8. Section through a cyst at the junction of graft roof (*g.r.e.*) and spread epithelium (*s.e.*). Note the difference in the size of the cells of the two epithelia, and that the basal cells of the graft roof epithelium stain more densely. Note also the difference in the tissues deep to the two epithelia. Toluidine blue. $\times 50$.
- Fig. 6. Autograft. Day 48. A plaque of bone is underlying spread epithelium, separated by a narrow band of connective tissue. Note the presence of bone canals containing blood vessels and poorly differentiated marrow cells. There is some lamination around the canals. Haematoxylin and eosin. $\times 75$.
- Fig. 7. Homograft. Day 12. A spicule of bone surrounded by periosteum, the osteogenic layer of which is well defined. Haematoxylin and eosin. $\times 350$.
- Fig. 8. Normal bladder epithelium giving a strongly positive reaction for alkaline phosphatase. Gomori technique, incubation time 30 min. $\times 550$.
- Fig. 9. Autograft. Day 10. Section showing difference between young fibroblasts (below) and osteoblasts (above). The osteoblasts show a greater degree of cytoplasmic basophilia. Toluidine blue. $\times 550$.
- Fig. 10. Normal bladder epithelium showing patchy distribution of alkaline phosphatase. Gomori technique, incubation time 24 hr. $\times 60$.

PLATE 2

- Fig. 11. Homograft. Day 6. The tissues immediately deep to the spread epithelium (*s.e.*) and to the graft itself give a positive reaction for alkaline phosphatase. The subepithelial tissues of the graft are negative. Gomori technique, incubation time 30 min. $\times 21$.
- Fig. 12. Autograft. Day 28. The endosteum and the osteogenic layer of the periosteum give a positive reaction for alkaline phosphatase. The osteocytes and the matrix are negative. Gomori technique, incubation time 30 min. $\times 108$.
- Fig. 13. Autograft. Day 48. The endosteum alone shows evidence of phosphatase activity. Gomori technique, incubation time 30 min. $\times 108$.
- Fig. 14. Autograft. Day 12. In the early stages of bone formation all elements give an intense reaction for alkaline phosphatase. Gomori technique, incubation time 30 min. $\times 108$.
- Fig. 15. Normal bladder epithelium removed at time of operation, showing glycogen in the superficial and middle layers of cells. P.A.S. technique. $\times 550$.
- Fig. 16. Normal bladder epithelium demonstrating increased cytoplasmic basophilia in the basal cells. Toluidine blue. $\times 550$.
- Fig. 17. Autograft. Day 3. The same epithelium as that illustrated in fig. 15 following implantation for 3 days. Note the decrease in the amount of glycogen. P.A.S. technique. $\times 550$.
- Fig. 18. Homograft. Day 12. Showing the presence of a bony plaque prior to the time of destruction of the epithelium. Note the presence of basophilia in the connective tissue underlying the graft and the spread epithelium, and its absence in the subepithelial tissues of the graft itself. Toluidine blue. $\times 29$.
- Fig. 19. Autograft. Day 5. Graft roof epithelium showing mitosis and cytoplasmic basophilia. Compare with the less active epithelium on the left. Toluidine blue. $\times 340$.
- Fig. 20. Autograft. Day 6. Spread epithelium with large cells and faint cytoplasmic basophilia. Compare with fig. 19 at same magnification. Toluidine blue. $\times 340$.





RIBONUCLEIC ACID-ALKALINE PHOSPHATASE DISTRIBUTION IN THE DEVELOPING TEETH OF THE RAT

By N. B. B. SYMONS

Dental School, University of St Andrews, Dundee

The presence of a cell, the kionoblast, morphologically different from the ameloblast, which is to be found in the internal enamel epithelium and ameloblast layers of the developing tooth, has recently been confirmed (Symons, 1955*a*), using material which had been fixed in 40 % chilled formalin. At the same time, the presence of a cell, the radial cell, amongst the odontoblasts of the developing tooth has also been confirmed. It was suggested that the kionoblast is related to the interprismatic substance of the enamel, and that the radial cell is related to the fibres of the organic matrix of the dentine. In view of this, it would seem that a re-interpretation of the existing descriptions of the distribution of alkaline phosphatase and ribonucleic acid in the developing tooth is necessary, since these descriptions have been based on the assumption that in the internal enamel epithelium and ameloblast layers there are only pre-ameloblasts and ameloblasts respectively, and that in the odontoblast layers there are only odontoblasts.

The distribution of ribonucleic acid is of interest since it is recognized to be of considerable importance in many aspects of cellular activity. On account of the elaboration of the organic matrices of dentine and enamel in tooth formation, a considerable content of ribonucleic acid in the cells of the developing tooth might be expected, as cells rich in ribonucleic acid may be taken to be actively engaged in protein manufacture (Caspersson, 1947). Since it is held that alkaline phosphatase may be concerned in the manufacture of ribonucleic acid (Bradfield, 1950) and in the production of fibrous proteins (Bradfield, 1950; Jeener, 1947), any correlation, in time of appearance or in localization, between ribonucleic acid and alkaline phosphatase could be significant due to the relationship probably existing between these two substances.

This relationship between ribonucleic acid and alkaline phosphatase in developing teeth has been examined by Johnson & Bevelander (1954), in which paper a useful account of previous work on nucleic acids in bone and tooth forming cells is given. Johnson & Bevelander made use of embryonic pig material for their investigation; in the present study the rat has been selected since that animal was used in a study of alkaline phosphatase distribution in developing teeth (Symons, 1955*b*) and in the demonstration of the kionoblasts and radial cells (Symons, 1955*a*). Moreover, as was pointed out in the former paper, the use of rat material for histochemical investigations of the developing tooth has the advantage that the significance of the morphological changes in the cells of the enamel organ has been carefully worked out, these changes having been correlated with the different phases of enamel formation (Marsland, 1951, 1952). Any alteration, therefore, in the distribution of ribonucleic acid or alkaline phosphatase activity in the enamel-forming cells which coincided with these phases would be particularly interesting.

MATERIALS AND METHODS

Ribonucleic acid distribution was studied in the developing teeth of rats from birth to 4 days after birth. The material was fixed in 40 % formalin at 3° C or in absolute alcohol and subsequently embedded in paraffin wax by routine methods. None of this material was decalcified. The pyronin-methyl green method of Trevan & Sharrock (1951), as modified by Pearse (1953), was used for the demonstration of ribonucleic acid. The presence of ribonucleic acid was checked by the use of ribonuclease on control section before staining with pyronin-methyl green (Pl. 1, figs. 1, 2). The description of the distribution of alkaline phosphatase activity is taken from the material used in a previous paper (Symons, 1955*b*). Some material fixed in 40 % chilled formalin was decalcified in 5 % nitric acid in 70 % alcohol for the purpose of obtaining a morphological picture for comparison.

OBSERVATIONS

Ribonucleic acid

In the internal enamel epithelium the kionoblasts show a marked basophilia with pyronin, considerably greater than that of the other, pre-ameloblastic, cells of this layer (Pl. 1, fig. 3). As the internal enamel epithelium changes into the fully differentiated ameloblast layer and enamel matrix formation commences, so the degree of basophilia shown by this layer becomes greater. This is mainly produced by an increased basophilia of the cytoplasm which is found in the broad distal zone of the ameloblast, that is between nucleus and forming enamel. The narrow basal zone of the cytoplasm shows a relatively faint basophilia. The kionoblasts show a less marked increase in cytoplasmic basophilia; they are thus somewhat less sharply differentiated from the ameloblasts than from the pre-ameloblasts in pyronin stained sections. At this stage the kionoblasts also show a greater basophilia in the distal zone than in the basal zone of cytoplasm. The cells of the stratum intermedium and the other cells of the enamel organ show only a faint cytoplasmic basophilia (Pl. 1, fig. 4).

The cells of the pulp (dental papilla) show a considerable cytoplasmic basophilia, before the appearance of an odontoblast layer, but after this has been established the reaction with pyronin becomes weaker. From their first appearance the cells of the odontoblast layer show a marked cytoplasmic basophilia, though that of the radial cells is heavier than that of the odontoblasts (Pl. 1, fig. 3). The basophilia to be seen in the odontoblast layer as a whole is of about the same intensity as that of the distal zone of the cells of the ameloblast layer. The basophilia of the cells of the odontoblast and ameloblast layers is only equalled, in the jaw region, by that of the cytoplasm of the osteoblasts and the cells of the basal layers of the skin and oral mucous membrane.

In the molar teeth and over most of the incisors, in the stages examined, the pattern of the cellular basophilia is as described. However, towards the tip of the incisor teeth, where the cells of the ameloblast layer have become reduced in length in association with the maturation of the enamel, an alteration is found in the basophilia shown by these cells. The deep basophilia of the distal zone of cytoplasm

is now confined to only part of this zone near the enamel surface, while the narrow basal zone shows marked basophilia (Pl. 1, fig. 7). This basophilia of the distal zone of the shortened ameloblasts corresponds to the basophilia shown by the highly columnar cells of the ameloblast layer which is particularly intense close to the end of the distal zone next to the enamel surface, especially in the later stages of enamel matrix formation.

The above description of the basophilia with pyronin shown by the developing tooth is based almost entirely on the formalin-fixed material. In material fixed in absolute alcohol, though the kionoblasts and radial cells are not cytologically distinguishable, yet their presence appears to be indicated by dark basophilic streaks which traverse the ameloblast and odontoblast layers (Pl. 1, fig. 1).

Alkaline phosphatase

Before differentiation of an odontoblast layer commences, the developing tooth shows some enzyme activity in the pulp (dental papilla) and to a greater extent in the stratum intermedium of the enamel organ. With the appearance of an odontoblast layer, heavy alkaline phosphatase activity is shown by a band of pulp tissue immediately deep to the odontoblasts. Less activity is shown by the odontoblast layer as a whole; but along the course of the von Korff fibres there is heavy alkaline phosphatase activity. An increased degree of enzyme activity also appears in the enamel organ, most heavily in the stratum intermedium (Pl. 1, fig. 6). The cells of the internal enamel epithelium, however, show no enzyme activity apart from linear streaks which pass across the full width of the layer. These streaks are apparent from the time of the first differentiation of an odontoblast layer until dentine formation has begun. In sections cut transversely through the internal enamel epithelium, the streaks appear as dots.

During production of the enamel matrix, apart from their narrow basal region, no alkaline phosphatase activity appears in the cells of the ameloblast layer. With the reduction in length which is associated with the commencement of enamel maturation, the cells of the ameloblast layer begin to show alkaline phosphatase activity throughout the whole of their cytoplasm (Pl. 1, fig. 8).

DISCUSSION

The kionoblasts and the radial cells can readily be distinguished in sections stained to show the distribution of ribonucleic acid, as such material can be fixed in 40 % chilled formalin and embedded in paraffin wax. In sections treated to show alkaline phosphatase activity the kionoblasts and radial cells cannot be directly demonstrated, since if the material is fixed in absolute alcohol these cells are not seen, and if the material is fixed in formalin the enzyme is destroyed by the subsequent embedding in paraffin wax.

However, in sections showing alkaline phosphatase activity which have been cut transversely through the odontoblast layer, it can be seen that the odontoblasts show only slight enzyme activity, whereas surrounding them there is a pattern of heavy alkaline phosphatase activity which coincides with the morphological picture of the radial cells and the related von Korff fibres as seen in material fixed

in 40 % chilled formalin. That is to say, although the radial cells cannot be seen directly in absolute alcohol-fixed material, they are indicated by their heavy enzyme content. This has already been described and figured (Symons, 1955*a*).

In the same way, a comparison can be made between sections cut in the more usual plane through teeth at similar stages of development, one tooth fixed in 40 % chilled formalin and stained to give the cytological picture and another fixed in absolute alcohol and treated to show alkaline phosphatase activity. The distribution of heavy alkaline phosphatase activity in the odontoblast layer has a pattern exactly like that of the radial cells and the related fibres of von Korff (Pl. 1, figs. 5, 6). This distribution of heavy enzyme activity in the odontoblast layer was seen and described previously (Symons, 1955*b*), but it was then wrongly interpreted as being along the course of the von Korff fibres alone.

The same difficulty is found in deciding the relative distribution of alkaline phosphatase activity in the kionoblasts and ameloblasts, except that during formation of the enamel matrix (apart from their narrow basal region) no alkaline phosphatase activity is shown by the cells of the ameloblast layer and so in this phase the question hardly arises.

For a brief period prior to dentine formation the internal enamel epithelium shows linear streaks of alkaline phosphatase activity. These have already been interpreted by their form and arrangement as indicating the position of the kionoblasts; and the enzyme activity has been taken as being possibly related to the thickening of the basement membrane between pulp and enamel which occurs before dentine formation begins (Symons, 1955*a*).

During the period of enamel maturation such heavy alkaline phosphatase appears in the cells of the ameloblast layer that it is difficult to decide its exact distribution. It seems probable, however, that both ameloblasts and kionoblasts show enzyme activity throughout their cytoplasm.

In the developing tooth of the rat, a feature of the distribution of ribonucleic acid in the cells of the enamel organ is the inverse relationship which this bears to that of alkaline phosphatase. In the cells of the ameloblast layer this relationship appears to reach an intracellular level.

In the enamel organ the stratum intermedium, and to a less extent the cells external to it, show considerable alkaline phosphatase activity; these cells show only a faint basophilia with pyronin. In the highly columnar cells of the ameloblast layer, alkaline phosphatase activity is completely absent except in the narrow basal zone of cytoplasm; the distribution of ribonucleic acid is the reverse of this, marked basophilia being found in the distal part of the cells and a faint degree in the basal zone (Pl. 1, fig. 4). In the shortened cells of the ameloblast layer also there is indication of this inverse relationship. The ribonucleic acid in the distal part of the cytoplasm is confined to a band close to the enamel (Pl. 1, fig. 7); while alkaline phosphatase activity, though found throughout the cytoplasm of the cells at this stage when examined carefully, is found to be most marked in the distal zone close to the nucleus and immediately adjacent to the enamel (Pl. 1, fig. 8).

The alteration in the ribonucleic acid content of the shortened cells of the ameloblast layer, compared with that of the highly columnar cells of the ameloblast layer, is surely a reflexion of the change of function from a phase of enamel matrix

production to one of enamel maturation, a change which is indicated more obviously by the morphological alteration of the cells. The difference in alkaline phosphatase activity between the two forms of cells has been described previously (Symons, 1955*b*).

Since the formation of enamel matrix has ceased at the time of appearance of the shortened cells of the ameloblast layer, it would seem likely that the reduced ribonucleic acid content of these cells is, like their alkaline phosphatase activity, related to the final heavy calcification of the enamel occurring during the maturation phase.

It has been shown (Symons, 1955*a, c*) that the radial cells are responsible for the formation of the fibres of the dentine matrix, apart from the element contributed by the von Korff fibres. It has been found in this work that though the odontoblasts possess only a slight alkaline phosphatase activity they have a very marked content of ribonucleic acid, which suggests that the odontoblasts are active cells.

It is pertinent therefore to consider what role the odontoblasts do play. There would seem to be four possibilities: (*a*) they may be concerned in the formation of the ground substance around the fibres of the dentine matrix; (*b*) they may be concerned in the calcification of the ground substance; (*c*) they may in some way subserve the transmission of sensory stimuli from the dentine to the pulp; (*d*) they may be related to the nutrition of the formed dentine. The first two possibilities are not mutually exclusive; indeed one might expect them to be closely related.

It would probably be helpful if some of the dietary deficiency experiments on the development of dentine were repeated. From these, further information might be obtained regarding the respective functions of the radial cells and the odontoblasts, depending upon which cell was most involved in the various circumstances.

SUMMARY

1. The distribution of ribonucleic acid in the developing teeth of rats from birth to 4 days after birth is described.
2. Previous findings on the distribution of alkaline phosphatase activity in the developing tooth have been re-interpreted, in view of the confirmation of the presence of kionoblasts and radial cells.
3. The pattern of cytoplasmic ribonucleic acid distribution in the enamel organ bears an inverse relationship to that of alkaline phosphatase activity; in the ameloblast, this reaches an intracellular level.
4. The cells of the ameloblast layer associated with the phase of enamel matrix formation show a different cytoplasmic distribution of ribonucleic acid compared with the cells of the ameloblast layer associated with enamel maturation.

I am greatly indebted to Dr E. W. Bradford for his helpful criticism of the manuscript.

REFERENCES

- BRADFIELD, J. R. G. (1950). The localization of enzymes in cells. *Biol. Rev.* **25**, 113-157.
CASPERSSON, T. (1947). *Symp. Soc. exp. Biol.* **1** (*Nucleic Acid*), 127-151. Cambridge University Press.
JEENER, R. (1947). Cytochemical effects of oestradiol. *Nature, Lond.*, **159**, 578.

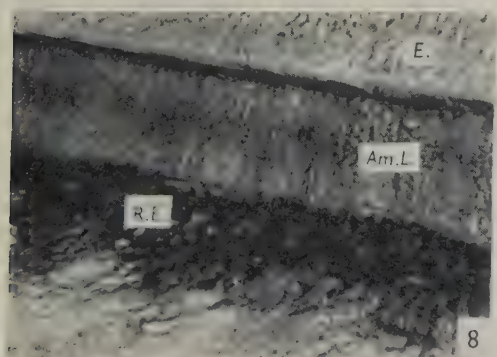
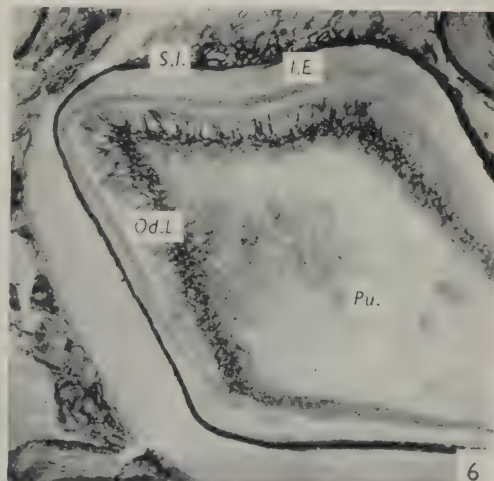
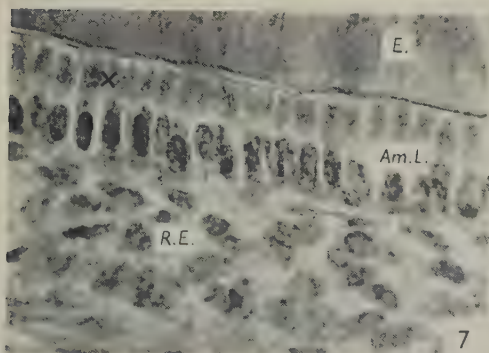
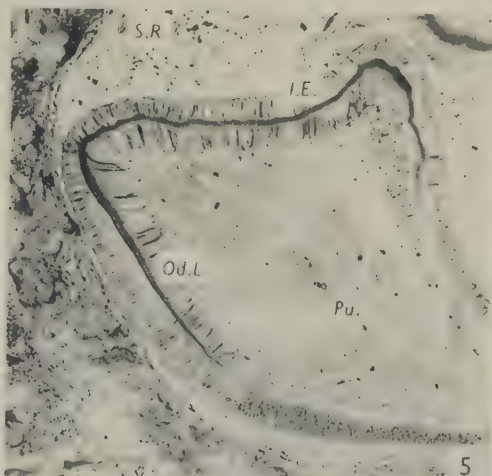
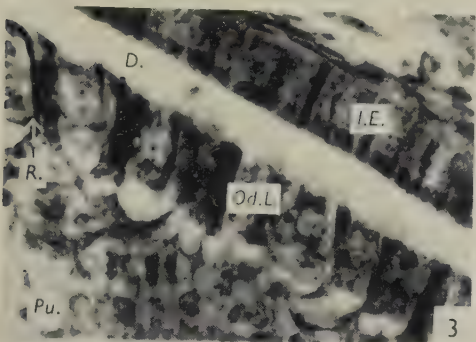
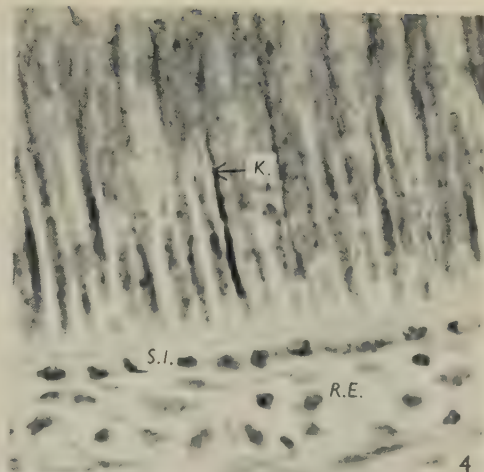
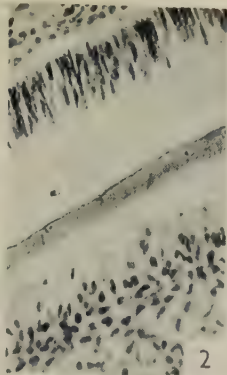
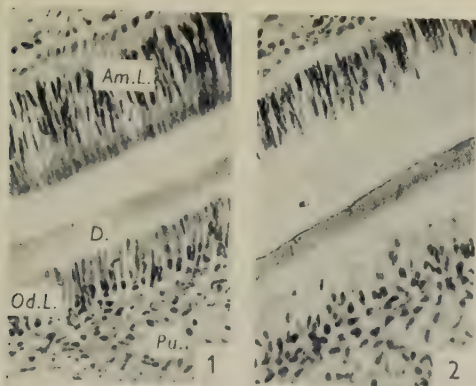
- JOHNSON, P. L. & BEVELANDER, G. (1954). The localization and interrelation of nucleic acids and alkaline phosphatase in the developing tooth. *J. dent. Res.* **33**, 128-135.
- MARSLAND, E. A. (1951). A histological investigation of amelogenesis in rats. I. Matrix-formation. *Brit. dent. J.* **91**, 251-261.
- MARSLAND, E. A. (1952). A histological investigation of amelogenesis in rats. II. Maturation. *Brit. dent. J.* **92**, 109-119.
- PEARSE, A. G. E. (1953). *Histochemistry*. London: Churchill.
- SYMONS, N. B. B. (1955*a*). The cells of the odontoblast, ameloblast and internal enamel epithelial layers. *Brit. dent. J.* **98**, 273-280.
- SYMONS, N. B. B. (1955*b*). Alkaline phosphatase activity in the developing teeth of the rat. *J. Anat., Lond.*, **89**, 238-245.
- SYMONS, N. B. B. (1955*c*). *Brit. dent. J.* In the press.
- TREVAN, D. J. & SHARROCK, A. (1951). A methyl green pyronin-orange G stain for formalin-fixed tissues. *J. Path. Bact.* **63**, 326-329.

EXPLANATION OF PLATE

List of abbreviations

<i>Am.L.</i>	Ameloblast layer	<i>Pu.</i>	Pulp
<i>D.</i>	Dentine	<i>R.</i>	Radial cell
<i>E.</i>	Enamel	<i>S.I.</i>	Stratum intermedium.
<i>I.E.</i>	Internal enamel epithelium	<i>R.E.</i>	Reduced enamel organ
<i>K.</i>	Kionoblast	<i>S.R.</i>	Stellate reticulum
<i>Od.L.</i>	Odontoblast layer		

- Fig. 1. 4-day-old rat. First lower molar, in the region of the highly columnar cells of the ameloblast layer and early enamel matrix formation. Part of the separation between the ameloblasts and the dentine is due to artefact. Fixed in absolute alcohol. Pyronin methyl-green stain. $\times 190$.
- Fig. 2. 4-day-old rat. A comparable section to that shown in fig. 1, but digested with ribonuclease before staining with pyronin methyl-green. Fixed in absolute alcohol. $\times 190$.
- Fig. 3. 4-day-old rat. Lower second molar, showing early dentine formation. The kionoblasts show more basophilia than the other cells of the internal enamel epithelium, and the radial cells more than the odontoblasts. Fixed in 40 % chilled formalin. Pyronin methyl-green stain. $\times 530$.
- Fig. 4. 4-day-old rat. Enamel organ of lower incisor showing the highly columnar cells of the ameloblast layer associated with enamel matrix formation. Note the marked basophilia of the distal zone compared with that of the narrow basal zone of the cells. Pyronin methyl-green stain. Fixed in 40 % chilled formalin. $\times 580$.
- Fig. 5. 3-day-old rat. Cusp of lower first molar. The pattern produced by the dark staining radial cells contrasts with the lighter staining odontoblasts. Similarly, the kionoblasts are evident amongst the other cells of the internal enamel epithelium. Fixed in 40 % chilled formalin. Decalcified. Masson. $\times 70$.
- Fig. 6. 3-day-old rat. Cusp of lower first molar. The distribution of alkaline phosphatase activity in the odontoblast layer very closely parallels the pattern of the radial cells as seen in fig. 5. Fixed in absolute alcohol, α -naphthyl phosphate and the diazonium salt of 5-chloro-*o*-toluidine. $\times 70$.
- Fig. 7. 4-day-old rat. Enamel organ of lower incisor showing the shortened cells of the ameloblast layer associated with enamel maturation. Note the altered cytoplasmic basophilia of these cells compared with that shown in fig. 4. \times indicates the position of the band of basophilia which persists in the distal zone. Pyronin methyl-green stain. $\times 580$.
- Fig. 8. 4-day-old rat. A comparable section to that of fig. 7 showing alkaline phosphatase activity in the cells of the enamel organ. Note the inverse relationship between the pattern of alkaline phosphatase distribution and that of the cytoplasmic basophilia (ribonucleic acid) shown in fig. 7. Absolute alcohol fixed, paraffin section, α -naphthyl phosphate and the diazonium salt of 4-chloro-*o*-anisidine. $\times 580$.



THE EARLY STAGES OF VAGINAL DEVELOPMENT IN THE SHEEP

By D. BULMER

Department of Anatomy, University of Cambridge

In a previous publication (Bulmer, 1952) it was shown that, in the development of the vagina of the sheep, a larger upper segment is formed from the Müllerian utero-vaginal canal, while the much shorter lower vaginal segment is of non-Müllerian origin. There was at that time insufficient early developmental material available to determine precisely the origin of this lower segment, and it is hoped that this gap is to a large extent filled by the series of foetuses which is described here.

Previous investigations of vaginal development in the Ungulata have been restricted to a few forms. Tourneux (1888), working on the horse, considered that a short lower vaginal segment was formed by the fusion together of the lower ends of the Müllerian and Wolffian ducts, in a similar manner to that which Tourneux & Legay (1884) found in the human subject. In the pig, Henneberg (1922) described the splitting of the upper end of the urogenital sinus by the downgrowth of a frontal septum, the dorsal segment forming the lower end of the vagina. Baxter (1934), on the other hand, stated that the lower vaginal segment of the pig arose from a median canal, formed by the fusion of the lower ends of the two Wolffian ducts. Kempermann (1934), working on the same form, found the lower vaginal segment to originate as an upgrowth from the dorsal wall of the urogenital sinus. It is at any rate clear that, in those ungulates in which vaginal development has been studied, there has been fairly general agreement on the non-Müllerian origin of the lower end of the vagina.

MATERIAL AND METHOD

Six female sheep foetuses were investigated, of between 32 and 70 mm. crown-rump length. In the younger specimens the whole pelvis was sectioned serially, while in the older ones the urogenital system was first dissected out. The material was fixed in formol-saline or in Bouin's fixative, embedded in paraffin and sectioned at 5-7 μ . The sections were stained with haematoxylin and eosin, haematoxylin and orange G, with or without eosin, or by a trichrome stain, in an attempt to obtain the maximal differentiation between the various types of epithelium involved.

DESCRIPTION AND MATERIAL

32 mm. foetus

By this stage the Müllerian ducts already extend down to the dorsal wall of the urogenital sinus. They are fused together for a short distance in the middle of the genital cord, but are separated from each other caudally as solid epithelial cords. Each 'Müllerian cord' consists of a mass of polygonal cells, whose cytoplasm stains

rather deeply with eosin, lying in close apposition with the medial aspect of the corresponding Wolffian duct, a considerably larger structure with a clearly marked cubical epithelium. The urogenital sinus is lined by three or four layers of very pale-staining polygonal cells, with the nuclei rather more closely packed in the basal layer than in the superficial layers. The Wolffian ducts enter the dorsal aspect of the upper end of the sinus by wide funnel-shaped openings, so that in transverse section the sinus appears to have two wide dorso-lateral 'Wolffian bays' (Pl. 1, fig. 1). The cubical Wolffian epithelium enters these bays, and there meets the sinus epithelium in a clear line of demarcation. Between the Wolffian bays the dorsal wall of the sinus is lined by the characteristic pale-staining stratified epithelium. As the sinus is followed caudally through the serial sections the Wolffian bays gradually diminish in size, and the Wolffian epithelium in their walls becomes increasingly replaced by sinus epithelium. At the lower limits of the Wolffian bays the dorso-ventral diameter of the sinus is considerably reduced, so that below this level it has a transversely elongated appearance in cross-section. It has been noted above that the two solid Müllerian cords are lying in the lower portion of the genital cord, in close association with the medial sides of the Wolffian ducts. This position is maintained as they are followed caudally, and where the Wolffian ducts enter the sinus the Müllerian cords run ventrally, medially and caudally, in relation with the medial aspects of the Wolffian bays. In this way they reach the median portion of the dorsal wall of the sinus which is lined by the typical sinus epithelium, from which their cells can be clearly differentiated.

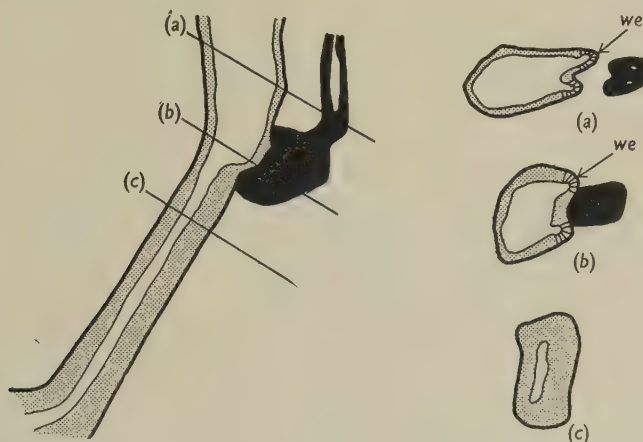
36 mm. foetus

The medial walls of the two Müllerian ducts are now fused with each other in the genital cord, but their lumina are still separated. The lower end of the Müllerian tissue forms a single solid cord of epithelial cells in contact with the dorsal wall of the urogenital sinus, and has lost its close association, noted in the 32 mm. embryo, with the medial aspects of the Wolffian ducts. The Wolffian bays of the sinus are again prominent structures (Text-fig. 1), and in them the cubical Wolffian epithelium meets the sinus epithelium in a clear line of demarcation (Pl. 1, fig. 2). Between the Wolffian bays, across the mid-line, the dorsal wall of the sinus is lined by the characteristic pale-staining stratified epithelium, and it is with this area that the Müllerian cells are in contact. On the right side the Wolffian bay extends down, with a small area of Wolffian epithelium in its dorsal wall, to the caudal limit of the Müllerian tissue, but on the left it disappears a short distance above this level. More caudally the sinus is transversely elongated in cross-section, and immediately below its area of contact with the Müllerian cells the epithelium of the dorsal sinus wall is considerably thickened (Pl. 1, fig. 3).

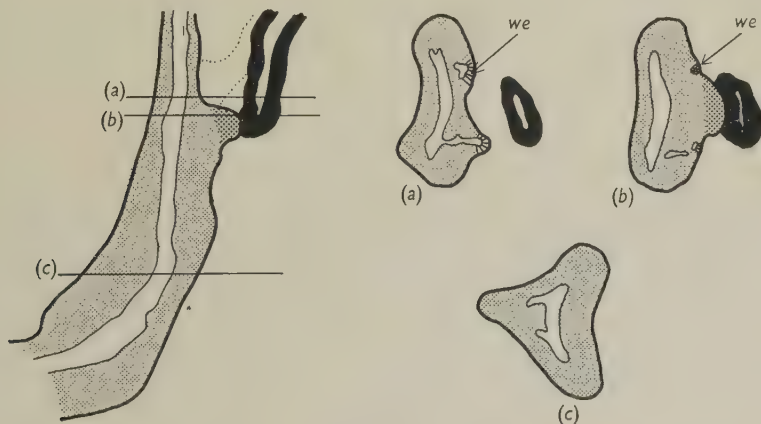
50 mm. foetus

The Müllerian ducts are now fused together in the genital cord to form a single utero-vaginal canal, lined by two or three layers of tightly packed and deeply staining columnar cells. The lower end of the utero-vaginal canal is transversely elongated, and its caudal tip divides into two short Müllerian bulbs. The Wolffian

ducts are still large structures in the genital cord, but the Wolffian bays by which they enter the sinus are much less prominent than in the younger specimens (Text-fig. 2). As the sinus is followed caudally the Wolffian bays soon disappear, though a small island of cubical Wolffian epithelium is continued downwards for a short distance in the dorsal wall of the sinus on each side (Text-fig. 2*b*). Apparently,



Text-fig. 1. 36 mm. foetus. The diagram on the left is a graphic reconstruction of a median sagittal section through the pars pelvina of the urogenital sinus, and its junction with the lower end of the Müllerian epithelium. (a-c), on the right, are transverse sections at the levels indicated. The Müllerian epithelium is shown by the solid black shading, while the stippling represents the sinus epithelium. The small cross-hatched areas, *we* in (a) and (b), show the extent of the Wolffian epithelium in the 'Wolffian bays'. The ventral aspect of the sections is towards the left.



Text-fig. 2. 50 mm. foetus. Graphic reconstruction of a median sagittal section through the sinus and the lower end of the utero-vaginal canal, with the corresponding transverse sections. The fine dotted line in the reconstruction indicates the position of the openings of the Wolffian ducts into the sinus, on either side of the plane of the section. The thinner stippling represents the typical sinus epithelium, while the heavier stippling shows the proliferation of darkly staining cells from the dorsal wall of the sinus. Other shading and lettering as for Text-fig. 1.

therefore, the Wolffian bays of the earlier stages have been partly absorbed into the dorsal wall of the sinus, so that only at their cranial extremities, where they are joined by the Wolffian ducts themselves, do they form actual projections from the sinus. This cranial recession of the Wolffian bays is so marked that they come to lie above the region where the Müllerian and sinus cells are in contact with each other. The island of Wolffian cells in the sinus wall below the Wolffian bays lies lateral to this area of contact, but ends some distance above its caudal limit.

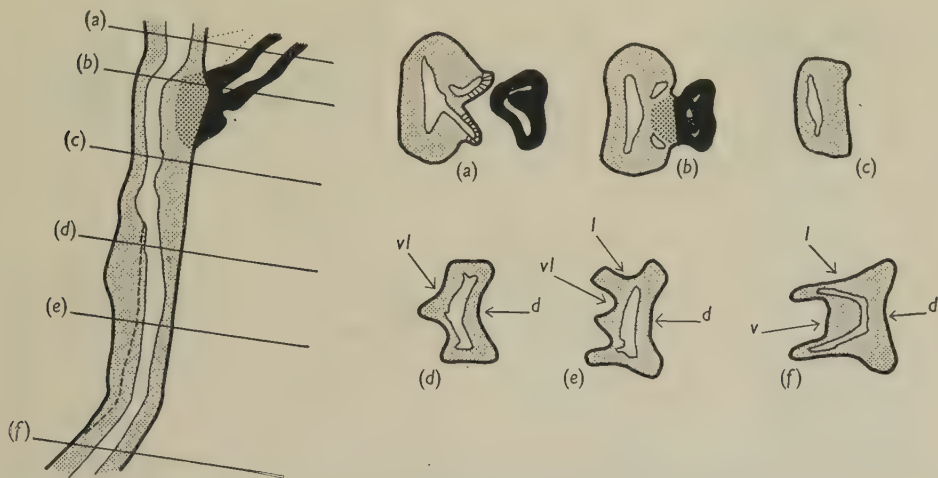
Because of the relative insignificance of the Wolffian bays, the dorso-ventral diameter of the upper part of the pars pelvina of the sinus is much less than in the younger specimens. At the same time the sinus epithelium is now much thicker, consisting of eight or nine layers of polygonal cells. The basal layers stain more deeply than the very pale-staining superficial layers, mainly because of the greater close-packing of their nuclei. The thickening of the sinus epithelium is most pronounced in its dorsal wall, immediately below the caudal limit of its contact with the Müllerian cells.

There is at this stage a considerable difference from the younger specimens in the appearance of the junction between the Müllerian and sinus epithelia (Pl. 1, figs. 4, 5). Separating the lumen of the utero-vaginal canal from that of the urogenital sinus is a solid mass of cells, and this may be divided into three zones. Immediately dorsal to the sinus lumen is an area of pale-staining polygonal cells, similar in appearance to the cells of the superficial layers of the rest of the sinus epithelium. Dorsally, the ventral wall of the utero-vaginal canal is formed by three or four layers of deeply staining polygonal cells, with very closely packed nuclei. Between these two zones is an intermediate layer, where the cells stain rather more deeply than the superficial cells of the sinus epithelium, but less deeply than those of the Müllerian epithelium. While it is difficult to be certain of the origin of these intermediate cells they appear to be more distinct from the Müllerian epithelium dorsally than from the sinus cells ventrally, and to be continuous with the deeply staining basal layers of the rest of the sinus epithelium. Their continuity with the sinus tissue is most clearly seen in Pl. 1, fig. 5, where the plane of section passes through the cranial end of this region. This cellular mass appears, therefore, to represent a proliferation of the basal layers of the sinus epithelium in the region of their contact with the Müllerian tissue, and in the median plane it encloses a small mass of mesoderm (Pl. 1, fig. 4). This suggests that it may originally have a double root from the sinus wall, arising on either side of the mid-line. By the fusion of these two roots dorsally, the enclosure of the small mesodermal septum may be explained. The Wolffian cells in the dorsal sinus wall lie lateral to this proliferation from the sinus and do not extend down to its caudal limit, so that there seems to be no possibility of their contributing to it.

58 mm. foetus

At this stage there is some change in the configuration of the urogenital sinus, and the lower end of the pars pelvina is marked by four inwardly-projecting longitudinal folds (Text-fig. 3*f*)—one dorsally, one ventrally, and one on either side. As the ventral fold is followed cranially it can be seen to split into two ventro-lateral folds (Text-fig. 3*e*), enclosing between them a ventral bay of the sinus. When the

longitudinal folds are traced upwards from this level they are found to become gradually less pronounced, and to disappear below the level of the contact between the Müllerian and sinus epithelia (Text-fig. 3c). Here the outline of the sinus shows little change from the 50 mm. stage, except that its transverse elongation is now less marked. The Wolffian bays are again insignificant projections from the dorsal aspect of the sinus, present only at the level of the Wolffian openings and lying at the cranial limit of the area of junction between Müllerian and sinus tissues (Text-fig. 3a). This junction, however, extends down to a lower level than the small tracks of Wolffian epithelium which are continued for a short distance in the dorsal wall of the sinus below the actual Wolffian bays.

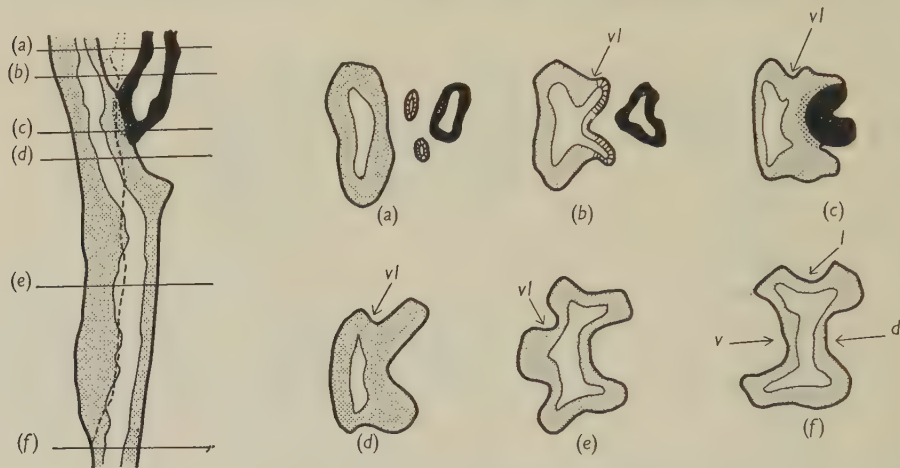


Text-fig. 3. 58 mm. fetus. Graphic reconstruction of a median sagittal section, with the corresponding transverse sections, through the sinus and the lower end of the utero-vaginal canal. The interrupted line in the reconstruction shows the relative position of the dorsal margin of the ventro-lateral fold, lying in the lateral wall of the sinus. In (d-f) the folds in the sinus wall are indicated as follows: *v*, median ventral fold; *d*, median dorsal fold; *l*, lateral fold; *vl*, ventro-lateral fold. Other shading as for Text-fig. 2.

Sections through this region are similar to those in the 50 mm. fetus, but the proliferation of sinus cells now stains more deeply and is therefore less easily distinguishable from the Müllerian cells dorsally (Pl. 2, fig. 8). Nevertheless, just below the caudal limit of the Müllerian tissue the epithelium of the dorsal wall of the sinus is considerably thickened, largely because of an increase in the number of layers of the deeply staining basal cells (Pl. 2, fig. 6). As this thickening is followed cranially, it is seen to become continuous with the mass of deeply staining cells which forms the junction with the Müllerian tissue (Pl. 2, figs. 7, 8). In other words, these cells clearly appear to be derived from the deeply staining basal cells of the sinus epithelium in this region. In this specimen there is no suggestion of the enclosure of a mesodermal septum in the proliferation of sinus cells, which now extends uninterrupted across the mid-line.

64 mm. foetus

In this specimen the system of longitudinal folds in the sinus wall shows further differentiation. The same folds occur in the lower part of the pars pelvina (Text-fig. 4*f*) as were noticed at the 58 mm. stage, and again the median ventral fold divides above to form two ventro-lateral folds (Text-fig. 4*e*). By this time, however, the ventro-lateral folds extend farther cranially, and as they are followed in this direction gradually incline dorsally around the lateral walls of the sinus (Text-fig. 4*d*). Eventually they become continuous with the folds which limit the lateral



Text-fig. 4. 64 mm. foetus. Graphic reconstruction of a median sagittal section, and corresponding transverse sections, through the pars pelvina and the lower end of the utero-vaginal canal. Lettering and shading as for Text-fig. 3.

aspects of the Wolffian bays from the dorsal wall of the sinus (Text-fig. 4*b*). Above this level the ventro-lateral folds meet each other around the cranial ends of the Wolffian bays, thus separating the Wolffian ducts from the sinus (Text-fig. 4*a*).

The slight constriction produced by the two ventro-lateral folds partially divides the upper part of the pars pelvina into dorsal and ventral portions, continuous with each other through the space between the two folds. The dorsal portion is continuous below with the caudal undivided part of the pars pelvina, and terminates cranially where it is joined by the Wolffian ducts. The ventral portion is widely continuous above with the urethra, but ends below where the two ventro-lateral folds meet each other to form the median ventral fold in the lower part of the pars pelvina. It can be seen from the diagrams in Text-fig. 4 that the lateral and dorsal folds of the sinus wall are continued into the lower end of the dorsal portion of the upper end of the pars pelvina. The lateral fold gradually disappears as it is followed cranially, but the dorsal fold persists, so that a transverse section through this part of the sinus (Text-fig. 4*d*) shows two wings of sinus tissue, projecting dorso-laterally. These 'dorso-lateral projections' are demarcated from the ventral portion of the sinus by the ventro-lateral folds, and from each other by the continuation of the dorsal fold. Further cranially (Text-fig. 4*e*), the dorsal wall of the sinus between

the two dorso-lateral projections is considerably thickened, and the dorsal fold is partially obliterated by the proliferation of darkly staining sinus cells, where it is in contact with the Müllerian tissue. At a still higher level (Text-fig. 4*b*), the dorso-lateral projections of the dorsal portion of the sinus are continuous with the Wolffian bays, by which the Wolffian ducts enter the sinus.

The proliferation of sinus cells arises from the medial aspects of the dorso-lateral projections, as well as from the dorsal wall of the sinus between them (Text-fig. 4*c*), though it appears to be rather less extensive than in the last specimen. Its cranial end again takes origin from the area of sinus epithelium which lies between the two Wolffian bays. The lumen of the Wolffian duct communicates with that of the cranial end of the ventral portion of the sinus, between the two ventro-lateral folds (Text-fig. 4*b*), but below this level the sinus lumen does not extend into the dorso-lateral projections of the dorsal portion of the sinus.

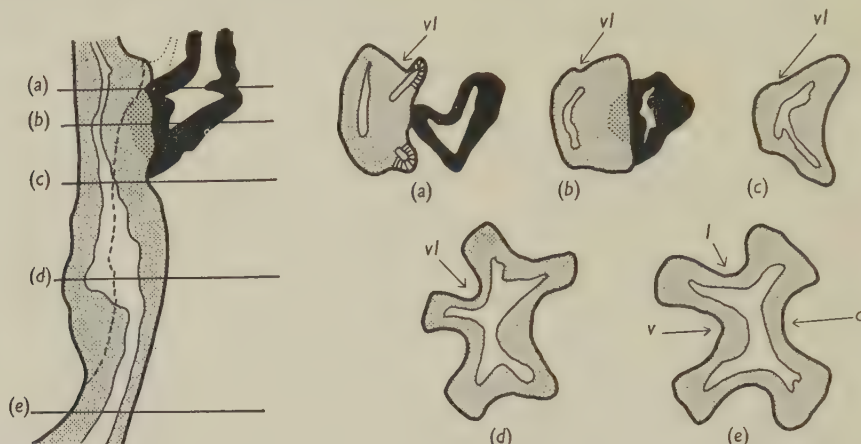
The dorso-lateral projections are of some interest, since it is these structures which, together with the sinus tissue joining them across the mid-line, now give rise to the proliferation of deeply staining sinus cells in contact with the caudal end of the Müllerian tissue. While they are continuous cranially with the Wolffian bays they are clearly not derived from them. It was noticed as early as the 50 mm. stage that the Wolffian bays became very insignificant structures, lying at the cranial limit of the junction of Müllerian and sinus epithelia. Their original caudal extensions beyond this level are apparently absorbed into the dorsal wall of the sinus, and this supposition is borne out by the presence of a small track of Wolffian epithelium on either side of the dorsal sinus wall, below the Wolffian bays. The dorso-lateral projections of the 64 mm. stage have arisen either by the ingrowth of the upper ends of the ventro-lateral folds, or by an active outgrowth of the sinus tissue behind them. While therefore they may incorporate some of the dorsal wall of the sinus into which the Wolffian bays were absorbed, the dorso-lateral projections are completely new structures, which only obtain their continuity with the Wolffian bays above by the extension of the ventro-lateral folds.

70 mm. foetus

Though the urogenital sinus and utero-vaginal canal of this foetus are rather larger than those of the 64 mm. specimen, there has been little developmental change between the two stages (Text-fig. 5). Again, the ventro-lateral folds may be said partially to divide the cranial part of the pars pelvina into dorsal and ventral portions, the upper end of the dorsal division forming two dorso-lateral projections which are continuous above with the Wolffian bays. The Wolffian ducts open into these bays, and a small lumen, lined by cubical epithelium, is continued downwards in the dorso-lateral aspect of the sinus on each side. The Wolffian duct, however, is no longer continuous with the lumen of the ventral division of the sinus, as it is in the 64 mm. foetus.

The Müllerian utero-vaginal canal lies in the genital cord between the two Wolffian ducts, which are still relatively large structures. At its lower end the utero-vaginal canal is rather elongated transversely, and lies in contact with the dorsal wall of the urogenital sinus. This contact is represented cranially by the

fusion of a ventral process of the utero-vaginal canal with the sinus epithelium which lies between the Wolffian bays (Text-fig. 5a). Farther caudally the ventral aspect of the Müllerian tissue fuses with the darkly staining proliferation from the dorsal wall of the sinus, which in this specimen completely fills the dorsal hollow between the dorso-lateral projections. Medially the Müllerian epithelium fuses with the sinus proliferation, while on each side it overlies the tip of the dorso-lateral projection (Text-fig. 5b; Pl. 2, fig. 9).



Text-fig. 5. 70 mm. foetus. Graphic reconstruction of a median sagittal section, and corresponding transverse sections, through the pars pelvina and the lower end of the utero-vaginal canal. Lettering and shading as for Text-fig. 3.

It might also be noted that in this foetus the rudiment of Bartholin's gland first appears, as a solid epithelial outgrowth from the bay of the sinus which extends dorso-laterally between the lateral and dorsal sinus folds, at the junction of the pars pelvina with the pars phallica.

DISCUSSION

It can be appreciated, from the material which has been described above, that several important developmental changes occur by the 70 mm. stage. The Wolffian bays become very insignificant structures, because of the absorption of their lower ends by the 50 mm. stage into the dorsal wall of the sinus. The track of Wolffian epithelium which runs down in the dorsal wall of the sinus in the 50 mm. foetus is very much smaller at the 70 mm. stage, so that the Wolffian epithelium is almost completely restricted to the actual openings of the Wolffian ducts. Associated with this, the main area of contact between the Müllerian and sinus epithelia comes to lie below the level of the Wolffian bays, but in the 70 mm. foetus the utero-vaginal canal still has a small area of contact cranially with the sinus epithelium between them. The epithelium of the dorsal wall of the sinus below the Wolffian bays shows signs of considerable activity during this period. Its thickness is increased, so that the lumen is excluded from this portion of the sinus. From the dorsal aspect there arises a proliferation of sinus cells which appears to displace the Müllerian epithelium dorsally, and by the development of the ventro-lateral folds, which obtain

their continuity above with the folds limiting the lateral margins of the Wolffian bays, this region can be identified by the 64 mm. stage as two dorso-lateral sinus projections. At the 70 mm. stage the dorsal hollow between the dorso-lateral projections is completely obliterated by the proliferation of sinus cells which was first identified at the 50 mm. stage. The distinction between the dorso-lateral projections and the Wolffian bays, which occupy a similar position in the younger foetuses, has already been emphasized.

The developmental changes which take place after this stage have been described earlier (Bülmer, 1952), but may be briefly summarized here. At the 80 mm. stage the division of the cranial portion of the sinus into ventral and dorsal elements is more pronounced, because of the accentuation of the ventro-lateral (or 'antero-lateral') folds. The dorsal division is in contact behind with the Müllerian epithelium, and in transverse section has the form of a triangular mass of sinus tissue. The proliferation of rather darkly staining cells from the dorsal sinus wall is very much less extensive, and is represented only by a thin layer of cells interposed between the Müllerian epithelium and the typical sinus tissue, but staining more deeply than the latter (Pl. 2, fig. 10). Into the cranial tip of the dorsal division of the sinus open the two Wolffian ducts, but their cubical epithelium is now completely restricted to the ducts themselves. The triangular mass which lies in contact with the Müllerian tissue appears to be derived both from the dorso-lateral projections and from the sinus proliferation of the earlier stages, and presumably the large part of the latter has been converted into, or replaced by, the typical pale-staining sinus epithelium. At the 98 mm. stage the region of the sinus which forms the junction with the Müllerian tissue has grown cranially as the free superior projection, behind, and separated from, the cranial tip of the dorsal division of the sinus which is joined by the Wolffian ducts. The mass of the sinus proliferation is now completely replaced by the pale-staining stratified sinus epithelium. Subsequently, by the 115 mm. stage, the further growth of the superior projection is associated with the elongation of the cranial extremities of the ventro-lateral folds, so that the cranial tip of the dorsal division of the sinus, with the attachments of the 'Wolffian cords', is also carried dorsally with the superior projection.

This method of development of the lower vaginal segment in the sheep does not closely resemble any of the earlier descriptions of vaginal development among the Ungulata. Baxter (1934), working on the pig, found that a lower vaginal segment was formed from the two 'dorso-lateral bays', the dilated openings by which the Wolffian ducts entered the sinus in the early stages of development. These bays were limited from the sinus on their ventro-lateral aspects by bilateral folds, which ran medially beneath the Wolffian bays to meet each other. Baxter suggested that the gradual fusion together of these folds, from above downwards, separated off a lower vaginal segment, whose cells then extended cranially on the ventral aspect of the caudal end of the utero-vaginal canal. Though the dorso-lateral bays were lined by a stratified epithelium Baxter considered that they represented the wide, funnel-shaped openings of the Wolffian ducts, and were therefore of Wolffian origin. On the other hand, Kempermann (1934) presented a very different account of the development of the lower vaginal segment in the pig, believing it to be a derivative of the urogenital sinus. He described the openings of the Wolffian ducts into the

sinus by means of the 'sinus bulbs', and the upgrowth of a lower vaginal segment from the urogenital sinus behind and between them. By its growth the lower vaginal segment carried with it, applied to its ventral aspect, the sinus bulbs, and it was continuous below with a longitudinal crest (or median dorsal bay) in the dorsal wall of the urogenital sinus—the crista urogenitalis posterior. Kempermann supposed, therefore, that the lower vaginal segment arose by an upward proliferation of the cranial end of the crista posterior—a structure which is not normally observed in the sheep embryo.

The dorso-lateral bays described by Baxter in the pig, and the sinus bulbs of Kempermann, appear to correspond with the structures which Mijsberg (1931), working on the bat, *Pipistrellus tralatitius*, termed the 'sinus horns'. These are the dilated passages by which the Wolffian ducts enter the sinus, and represent the remains of the earlier cloacal horns after the separation of the Wolffian and ureteric orifices from each other. Similar structures were observed by Baxter (1935) in the American opossum, and there found to give rise to the lower segments of the lateral vaginal canals. In this form, however, Baxter did not attribute a Wolffian origin to the sinus horn, and pointed out that it was lined by the characteristic sinus epithelium. In the sheep the Wolffian bay appears to represent the sinus horn, but it has been noted that the lower vaginal segment does not arise directly from it. The dorso-lateral projections do not correspond with the sinus horns, and their delimitation from the rest of the sinus is a result of the development of the longitudinal fold system of the sinus wall. It may be said that the cranial tip of the dorsal division of the sinus, into which open the Wolffian ducts, must incorporate some of the Wolffian bays or sinus horns, but it has been seen that this part of the dorsal division seems to play no part in the formation of the lower vaginal segment. There is no evidence of any participation of the Wolffian ducts in the lower vaginal segment, which is lined by an epithelium similar in type to that which occurs in the rest of the sinus.

Bergschicker (1912) considered that the lower vaginal segment of the cow was formed by a fusion of Müllerian and sinus epithelia. It is agreed that, during the formation of the sinus proliferation and the dorso-lateral projections in the sheep, there is such an intimate fusion of the Müllerian and sinus cells that it is extremely difficult to identify a hard and fast line of distinction between them in every section. Moreover, attempts to demonstrate a basement membrane separating them, using the modification of Long's reticular stain described by Millen & Woollam (1954), were unsuccessful. This is in contrast to the finding of Baxter in the pig. Nevertheless, the subsequent lining of the lower vaginal segment by the typical sinus epithelium seems to make the assumption of a Müllerian contribution to the lower vaginal segment of the sheep unjustifiable.

It is of some interest that in certain other mammals different workers have found the development of a sinus contribution to the vagina by the fusion of two dorso-lateral sinus projections, each arising in the neighbourhood of the Wolffian opening—e.g. Mijsberg (1931), Koff (1933), Raynaud (1942), Jost (1947), Carr (1953). In the sheep, the lower vaginal segment assumes a regular form through the fusion of the dorso-lateral projections with the proliferation of rather darkly staining cells from the dorsal sinus wall. This proliferation is of considerable interest since such a structure appears to have been described previously only in the human foetus,

where Vilas (1932) considered that it gave rise to the entire vagina. Its identity in the sheep seems to be established, and the appearances seen in Pl. 1, fig. 5, together with the later replacement of the darkly staining cells by the typical sinus epithelium, indicate its origin from the darkly staining basal cells of the sinus.

SUMMARY

1. The early stages in the development of the vagina and urogenital sinus are described from a series of six sheep foetuses. These are correlated with the findings in older specimens, recorded earlier.

2. The short lower vaginal segment arises from the urogenital sinus, and in its early development three components take part.

3. From the median portion of the dorsal wall of the sinus there arises, by the 50 mm. stage, a proliferation of rather darkly staining cells, apparently derived from the basal layers of the sinus epithelium.

4. By the 64 mm. stage, the dorsal portion of the sinus, which lies in contact with the caudal end of the Müllerian tissue, forms two 'dorso-lateral projections'. These are demarcated from the ventral portion of the sinus by longitudinal folds in the sinus wall, and they do not correspond with the 'sinus horns' described by other workers.

5. The fusion of the sinus proliferation with the two dorso-lateral projections forms the basis of the lower vaginal segment, which then extends further dorsally and cranially. There is no evidence of any contribution to the lower vaginal segment from Wolffian epithelium.

6. The relation is discussed between these findings in the sheep and those which previous workers have described in other forms.

I am most grateful to Prof. J. D. Boyd for his advice and encouragement. My thanks are also due to Mr R. F. Smith for technical assistance, and to Mr J. F. Crane for the photography.

REFERENCES

- BAXTER, J. S. (1934). Some observations on the development of the vagina in the pig. *J. Anat., Lond.*, **68**, 239-250.
- BAXTER, J. S. (1935). Development of the female genital tract in the American opossum. *Contr. Embryol. Carneg. Instn*, **25**, 15-35.
- BERGSCHICKER, A. (1912). Die Müllerschen und Wolffschen Gänge und die Bildung des weiblichen Genitaltrakts beim Rind. *Arch. Anat. Physiol., Lpz.*, **1**, 1-54.
- BULMER, D. (1952). Observations on the development of the lower end of the vagina in the sheep. *J. Anat., Lond.*, **86**, 233-245.
- CARR, E. B. (1953). The development of the rabbit vagina. *J. Anat., Lond.*, **87**, 423-431.
- HENNEBERG, B. (1922). Anatomie und Entwicklung der äusseren Genitalorgane des Schweines und vergleichend-anatomische Bemerkungen. *Z. ges. Anat.* **1**, *Z. Anat. EntwGesch.* **63**, 431-493.
- JOST, A. (1947). Recherches sur la différenciation sexuelle de l'embryon de Lapin. *Arch. Anat. micr. Morph. exp.* **36**, 151-200.
- KEMPERMANN, C. T. (1934). Beiträge zur Entwicklung des Genitaltrakts der Säuger. II. Die Entwicklung der Vagina des Hausschweines bis drei Tage nach dem Wurf. *Morph. Jb.* **74**, 221-261.
- KOFF, A. (1933). Development of the vagina in the human foetus. *Contr. Embryol. Carneg. Instn*, **24**, 59-91.
- MIJSBERG, W. A. (1931). Studien über die Entwicklung des weiblichen Genitaltractus bei den Säugern. IV. Die Entwicklung der Vagina und des Sinus urogenitalis beim *Pipistrellus tralaticus*. *Z. ges. Anat.* **1**, *Z. Anat. EntwGesch.* **96**, 183-214.

- MILLEN, J. W. & WOOLLAM, D. H. M. (1954). The reticular perivascular tissue of the central nervous system. *J. Neurol. Psychiat.* **17**, 286-294.
- RAYNAUD, A. (1942). Recherches embryologiques et histologiques sur la différenciation sexuelle normale de la Souris. *Bull. biol.* (Suppl.), **29**, 1-114.
- TOURNEUX, F. (1888). Sur la participation des canaux de Wolff à la constitution de l'extrémité inférieure du vagin chez le fœtus de Cheval. *C.R. Soc. Biol., Paris* (Ser. 8), **5**, 379-381.
- TOURNEUX, F. & LEGAY, C. (1884). Mémoire sur le développement de l'utérus et du vagin. *J. Anat., Paris*, **20**, 330-386.
- VILAS, E. (1932). Über die Entwicklung der menschlichen Scheide. *Z. ges. Anat.* **1**, *Z. Anat. EntwGesch.* **98**, 263-292.

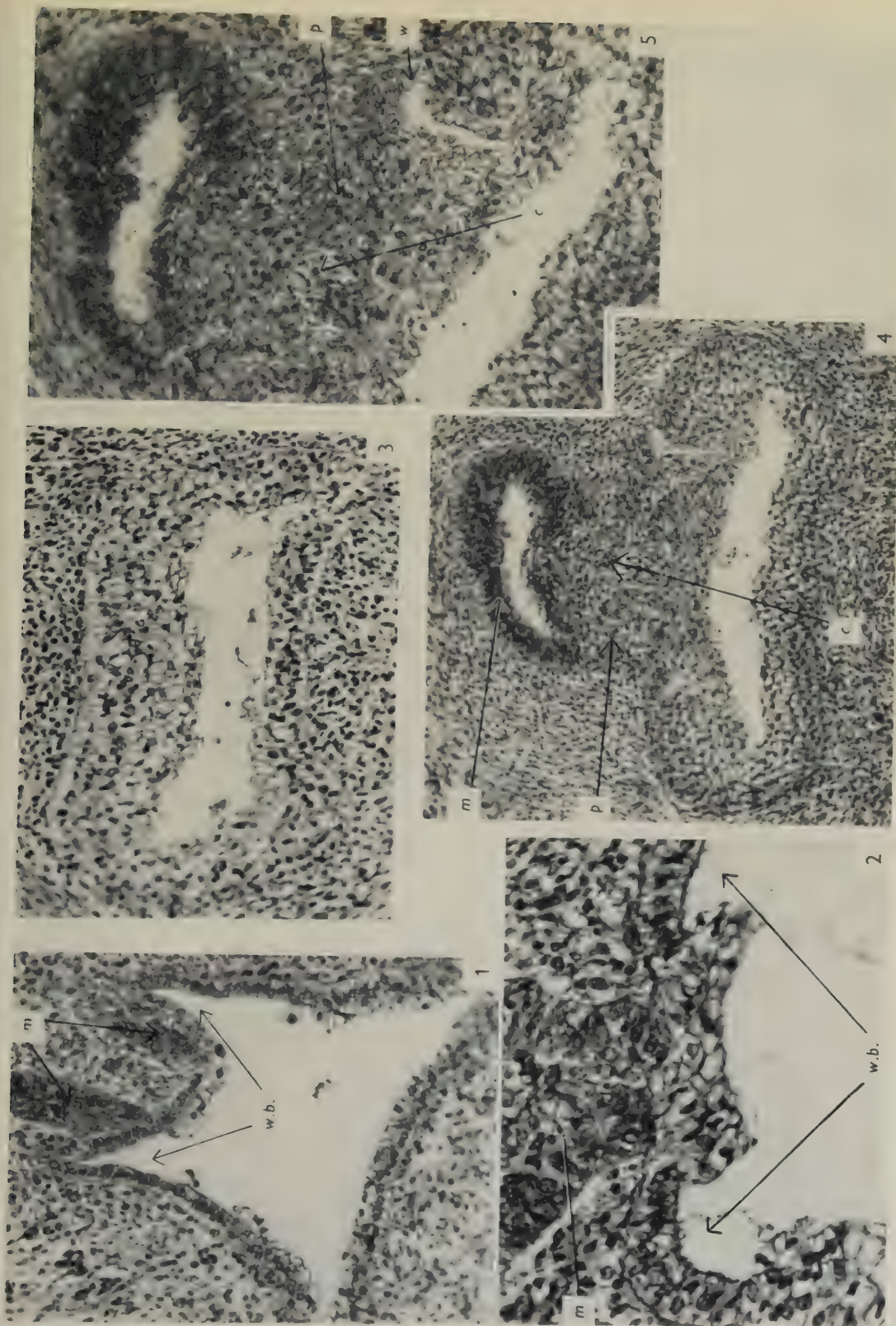
EXPLANATION OF PLATES

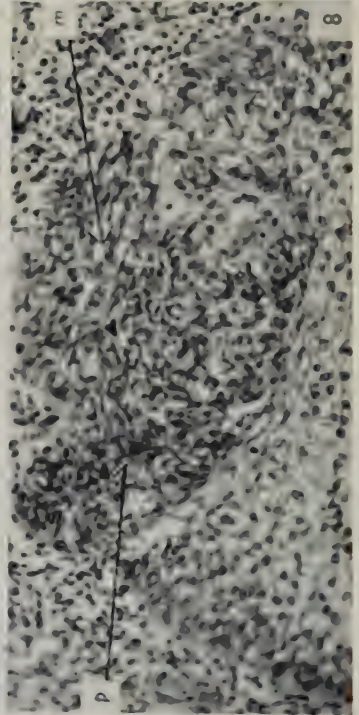
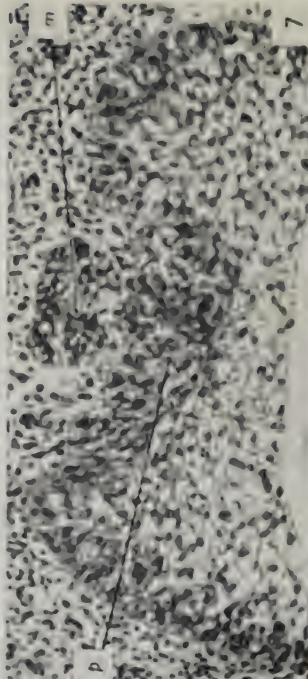
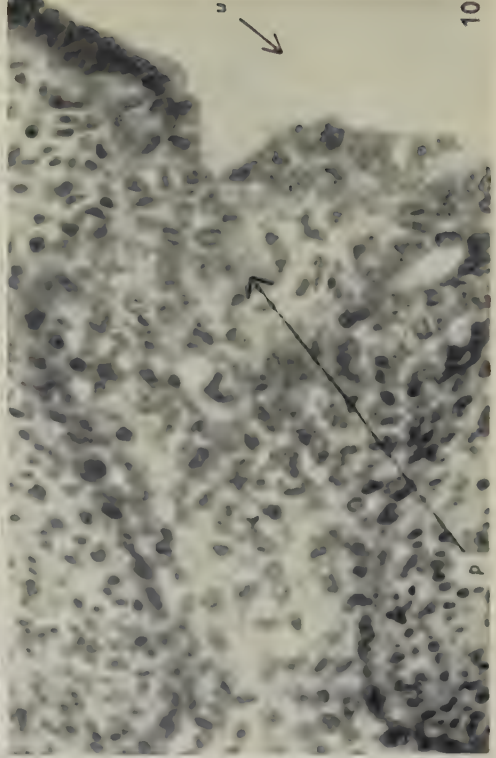
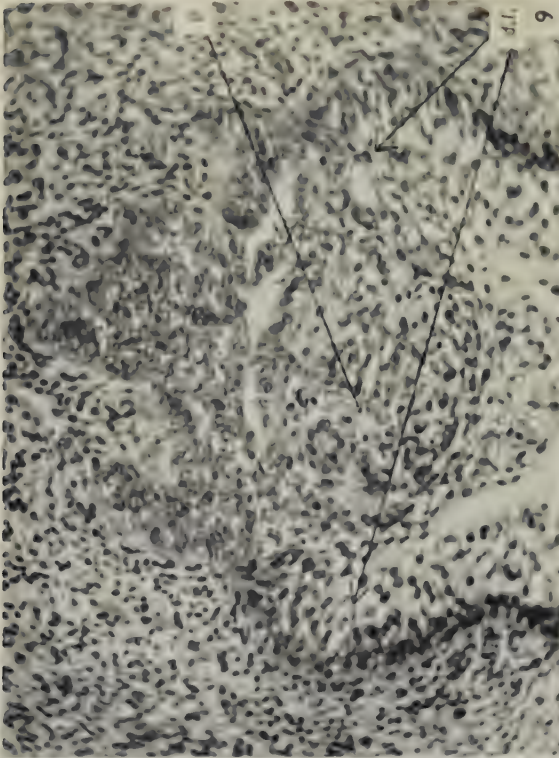
PLATE 1

- Fig. 1. 32 mm. foetus, showing a transverse section through the upper end of the urogenital sinus. The Wolffian bays (*w.b.*) are seen dorso-laterally, lined by a cubical epithelium, but the Müllerian cords (*m*) are in contact with the stratified sinus epithelium lining the dorsal sinus wall between them. ($\times 215$.)
- Fig. 2. Transverse section through the dorsal wall of the sinus in the 36 mm. foetus. The single Müllerian cord (*m*) is in contact with the stratified sinus epithelium and not with the cubical epithelium of the Wolffian bays (*w.b.*). ($\times 450$.)
- Fig. 3. 36 mm. foetus, showing a transverse section through the urogenital sinus immediately below its junction with the Müllerian epithelium. The thickening of the dorsal wall of the sinus, towards the top of the section, is clearly evident. ($\times 215$.)
- Fig. 4. Transverse section through the junction of the lower end of the utero-vaginal canal with the dorsal wall of the sinus in the 50 mm. foetus. The proliferation of rather intermediate-staining cells (*p*) separates the typical pale-staining sinus epithelium from the darkly staining Müllerian tissue (*m*). A small mesodermal mass (*c*) is embedded in the sinus proliferation, close to the dorsal wall of the sinus itself. ($\times 147$.)
- Fig. 5. 50 mm. sheep foetus. Transverse section through the cranial end of the sinus proliferation (*p*), which is again split by a small mesodermal septum (*c*). The continuity of the proliferation with the cells of the dorsal wall of the sinus can be clearly seen. A small island of Wolffian epithelium (*w*) lies in the dorso-lateral aspect of the sinus wall. ($\times 215$.)

PLATE 2

- Fig. 6. 58 mm. foetus, showing a transverse section through the sinus immediately below the caudal end of the Müllerian epithelium. Towards the top of the section the thickening of the dorsal wall of the sinus is evident, most marked in the basal layers of cells. ($\times 215$.)
- Fig. 7. 58 mm. foetus. Transverse section through the dorsal wall of the sinus, passing 49μ cranial to fig. 6. The darkly staining cells of the basal layers of the sinus epithelium now form the caudal end of the sinus proliferation (*p*), in contact with the lowermost tip of the Müllerian tissue (*m*). ($\times 215$.)
- Fig. 8. 58 mm. foetus. Transverse section through the dorsal wall of the sinus, passing 105μ cranial to the plane of fig. 7. The section now passes through the central mass of the sinus proliferation (*p*), which, though it can be distinguished from the Müllerian epithelium (*m*) dorsally, stains much more deeply than in the 50 mm. embryo. Comparison with figs. 6 and 7 shows that the proliferation is continuous with the thickening of the basal layers of the sinus epithelium seen in fig. 6. ($\times 215$.)
- Fig. 9. Transverse section near the middle of the sinus proliferation in the 70 mm. foetus. The lumen of the utero-vaginal canal is divided into three transverse slits, and the Müllerian epithelium is in contact ventro-laterally with the tips of the dorso-lateral sinus projections (*d.l.*). Across the median plane the Müllerian tissue lies on the sinus proliferation (*p*), which fills the space between the medial aspects of the dorso-lateral projections. ($\times 215$.)
- Fig. 10. High-power view of the junction of Müllerian and sinus epithelia in an 80 mm. foetus. The lumen of the utero-vaginal canal is shown at *u*. A narrow layer of rather intermediate-staining cells (*p*), representing the remains of the sinus proliferation, intervenes between the pale-staining sinus epithelium and the ventral wall of the utero-vaginal canal. ($\times 450$.)





HISTOCHEMISTRY OF THE RABBIT PLACENTA

By J. DAVIES*

Department of Anatomy, State University of Iowa, Iowa City, Iowa

The histochemistry of the rodent placenta has been the subject of extensive studies and reviews (Wislocki, Deane & Dempsey, 1946; Hard, 1946; Pritchard, 1947; Amoroso, 1952). These papers, however, give little detailed information on the rabbit placenta, the observations of Wislocki *et al.* in particular being restricted to the distribution of acid and alkaline phosphatases in a few specimens at term.

Accordingly, an investigation has been made of the rabbit placenta at numerous stages from about the 12th day to term with special reference to periodic acid-Schiff reacting substances, lipoids, cytoplasmic basophilic material and alkaline phosphatase. The findings are briefly discussed in relation to some outstanding problems of placental physiology.

MATERIAL AND METHODS

Uterine capsules of pregnant rabbits killed at timed intervals after observed copulation from the 12th day to term were sectioned in paraffin after appropriate fixation. For general histological studies Bouin fixation was used followed by staining with iron haematoxylin and the one-step trichrome method of Gomori (1950*a*). Periodic acid-Schiff (PAS) positive substances were identified in paraffin sections of Bouin-fixed material after the method given by Lillie (1953) but omitting the acid-reducing rinse; control sections were treated with saliva to eliminate glycogen before staining. Similar sections were stained with the aldehyde fuchsin method Gomori (1950*b*) with and without prior oxidation with potassium permanganate and sulphuric acid (Halmi & Davies, 1953). Cytoplasmic basophilia and metachromasia were studied in paraffin sections after Bouin fixation by staining in a solution of 1 % toluidine blue, diluted 1 in 100, for several minutes; sections were first examined in water, then dehydrated and mounted in the usual manner. Control sections were incubated in a solution of crystalline ribonuclease at a strength of about 1 mg. per ml. at pH 7. Alkaline phosphatase activity was demonstrated in paraffin sections after fixation in cold absolute alcohol using sodium glycerophosphate as a substrate (Gomori, 1939), the incubation time being in most cases kept under an hour to reduce diffusion. Lipoid materials were studied either in frozen sections of formalin-fixed material stained with Sudan black or in paraffin sections after fixation for several days in Flemming's fluid.

OBSERVATIONS

The general details of the placentation of the rabbit and the disposition of the foetal membranes have been described by many authors (Duval, 1892; Mossman, 1937; Amoroso, 1952) and will not be considered further. A transverse section through the

* John and Mary R. Markle Scholar in Medical Science.

uterine capsule at about the 13th day, stained with the PAS method, is illustrated in Pl. 1, fig. 1. The decidual tissues of the subplacental and paraplacental areas contain large amounts of glycogen, and there are isolated plaques of glycogen-containing tissue in the obplacental region. The trophoblast is free of glycogen. The lumen of the yolk sac is filled with a clear jelly-like material which is faintly PAS-positive and shows a faint pink metachromasia in toluidine blue preparations. The PAS reaction is unaffected by saliva and is probably due to mucoprotein or mucopolysaccharide. The outer wall of the yolk sac is atrophic; the inner wall, which is partially inverted, is well differentiated and made up of columnar cells the histochemical properties of which will be discussed fully at a later stage. Vascularization of the trophoblastic columns (Duval, 1892) of the allanto-placenta is not conspicuous at this stage, and the definitive characteristics of the placenta are not fully established until about the 17th day. The delayed vascularization of the allanto-chorion is a feature of the rabbit in contrast to such rodents as the rat and guinea-pig in which it is precocious.

Uterine stroma

Details of the glycogen-containing decidual cells of the subplacental region are shown in Pl. 1, fig. 2. The decidual cells of the intermediate zone of the subplacenta are perivascular in position in relation to the large sinuses; in the deeper part of the subplacenta, adjoining the uterine muscle, they are disposed in a continuous sheet and form the so-called 'zone of separation' (Lockhead & Cramer, 1908). The glycogen appears in particulate form in Bouin-fixed sections; in sections fixed in cold absolute alcohol it is diffusely distributed within the cells. The latter probably represents more truly its disposition in life. In the superficial zone of the subplacenta, in relation to the invading trophoblast, the decidua shows many areas of necrosis with concomitant liberation of intracellular glycogen. In later stages calcified masses may be identified in such necrotic areas.

A striking feature of the decidual stroma is the strong metachromasia of the ground substance (Pl. 1, fig. 3). The reaction involves not only the coarse connective tissue septa but also the delicate reticulum between the individual decidual cells. It is unaffected by treatment either with ribonuclease or hyaluronidase. The ground substance is also strongly PAS-positive (Pl. 2, fig. 5) and stains moderately with aldehyde fuchsin but intensely after preliminary oxidation with permanganate and sulphuric acid. These histochemical reactions, in so far as they are understood, probably represent deposits of acid mucopolysaccharide (Wislocki, 1951). The decidual stroma also shows marked alkaline phosphatase activity (Pl. 1, fig. 4; Pl. 2, fig. 7). The endothelial giant cells in the subplacental region are strongly basophilic (Pl. 1, fig. 3), the reaction being prevented by ribonuclease. They also show alkaline phosphatase activity (Pl. 1, fig. 4).

Decidual tissue, rich in glycogen, persists until term in the 'zone of separation' (Pl. 2, fig. 5). The ground substance stains maximally with the PAS procedure at this stage and also shows the aforementioned reactions with aldehyde fuchsin and toluidine blue. Between the decidual cells in this area and the placenta proper is a wide zone of necrotic material (Pl. 2, fig. 5) which is strongly PAS-positive.

The uterine epithelium

A feature of the epithelium of the pregnant rabbit uterus is its syncytial transformation in the middle third of gestation. This change is apparently a non-specific one and has been produced by the writer in the uterus of the pseudo-pregnant rabbit by passing a silk thread through the uterine wall. An early stage of this syncytial 'metaplasia' is shown in Pl. 2, fig. 6. The syncytial area shows in this preparation many intracellular droplets which are PAS-positive and may be glycoprotein in nature since they are unaffected by saliva. Similar PAS-positive droplets are found in the uterine epithelium elsewhere, but, being small and fine, are not well illustrated in Pl. 2, fig. 6. The PAS-positive reaction of the luminal margin ('brush border') of the epithelium and of the glandular secretions ('uterine milk') is also shown. All these features are well revealed in aldehyde fuchsin preparations only after oxidation as described above; indeed, under these circumstances, the staining procedure is almost identical with that of the PAS method.

The uterine epithelium is also strongly basophilic, this reaction being due in all probability to ribonucleoprotein, since it is abolished by ribonuclease treatment. Glycogen and alkaline phosphatase (Pl. 2, fig. 7) were not observed in the uterine epithelium at any stage.

The trophoblast

The allantois is illustrated in Pl. 2, figs. 8 and 9, as it dips down into the sulci between the placental cotyledons. The allantoic wall is well differentiated at about the 17th day, the epithelial lining consisting of cuboidal cells which have a tendency to form gland-like spaces (Pl. 2, fig. 9). The allantoic epithelium contains small amounts of glycogen, but shows no other significant histochemical properties.

The trophoblastic epithelium at all stages shows intense alkaline phosphatase activity (Pl. 2, fig. 8) and is also strongly basophilic (Pl. 3, fig. 10). The latter reaction is probably due to ribonucleoprotein since it is prevented by ribonuclease. The trophoblast also contains large amounts of intracellular fat (Pl. 3, fig. 11) in the form of discrete droplets. No attempt was made to investigate this fatty material with more refined methods.

Particular attention was paid to the placenta at term in view of the reports of the disappearance of the trophoblast in certain areas, producing a 'haemo-endothelial' condition (Mossman, 1937). In PAS preparations at term the trophoblast stains faintly while the basement membrane of the foetal capillaries stains intensely (Pl. 3, fig. 12). These staining reactions permit a careful study of the exact state of the placental membrane, especially in areas of extreme attenuation of the trophoblastic envelope. No evidence was found to support the concept of a 'haemo-endothelial' condition at any point even in post-mature placentae. Alkaline phosphatase preparations likewise lend no support to this view, since a thin sheet of syncytiotrophoblast may be identified at all points between the foetal vessels and the maternal blood spaces.

The yolk sac

The breakdown of the atrophic outer wall of the yolk sac takes place around the 15th day (Duval, 1892) and thus exposes the epithelium of the inner wall directly to the uterine secretions (Pl. 4, fig. 13). The latter, which constitute the so-called 'uterine milk', are creamy in consistency and are abundant in the middle third of gestation. In sections the uterine milk consists of a strongly acidophilic matrix, which also stains intensely with the PAS method and with aldehyde fuchsin after oxidation. It is not metachromatic. It appears to contain small quantities of glycogen especially in the early stages; however, this may be an artefact, caused by the mechanical displacement of the material from the adjoining decidual areas which are very rich in glycogen. The uterine milk contains much cellular and nuclear debris in addition to many multinucleate giant cells of trophoblastic origin (Duval, 1892). The latter appear to undergo degeneration and disintegration within the uterine lumen (Pl. 4, fig. 13) and may there furnish an important source of nucleoprotein. These giant cells continue to be budded off from the remains of the outer trophoblastic shell, the so-called 'trophoblastic fringe' (Duval), until a very late stage.

The yolk-sac epithelium is columnar and rests on a basement membrane and a highly vascular mesenchyme which is limited on its inner side by a layer of flattened cells lining the exocoelomic cavity (Pl. 4, fig. 13). The luminal margin of the cells, when not obscured by the masses of intracellular granules, shows a PAS-positive 'brush border' which also shows moderate alkaline phosphatase activity. The supranuclear parts of the cells are frequently distended by many hyaline droplets or granules which show many of the staining properties of the uterine milk, being strongly PAS-positive and acidophilic (Pl. 4, fig. 14). They are unaffected by saliva. Glycogen has not been demonstrated at any stage in the vitelline epithelium. In addition to the above staining reactions the droplets show a strong affinity for iron haematoxylin (Pl. 4, fig. 16). They also are resistant to decolorization in the Gram procedure and so may be termed Gram-positive or Gram-variable, since all of them do not retain the gentian violet. These reactions are similar to those given by the intracellular droplets observed in the proximal tubular epithelium of the kidney of rats excreting protein (Davies, 1954), where they have been interpreted as glycoprotein in nature. It is likely that the yolk-sac droplets represent protein material absorbed from the uterine milk.

The yolk-sac epithelium also shows marked cytoplasmic basophilia especially in the peri- and infranuclear regions of the cells. The material responsible for this reaction is probably ribonucleoprotein, since it is removed by ribonuclease. The yolk-sac epithelium also contains many discrete droplets of fat located in the basal part of the cell (Pl. 4, fig. 17).

In the late stages of gestation the wall of the yolk sac become villous, the basement membrane and the subepithelial stroma become thickened (Pl. 4, fig. 15). The cells become very tall and continue to show considerable numbers of PAS-positive droplets and fat. Cytoplasmic basophilia and alkaline phosphatase activity are markedly reduced in the last third of gestation.

DISCUSSION

Histochemically the rabbit placenta shows many points of similarity with that of other rodents. The placentation of the rabbit differs, however, in the superficial character of the implantation and the delay in the establishment of a vascular allantochorion. This delay results in a relative prolongation of the 'histotrophic' phase of embryonic nutrition during which the activity of the yolk sac in the absorption and synthesis of many important materials may be of great significance. These materials include proteins, nucleoproteins, fat, and possibly iron. The amniotic water may also be derived from the vital activity of the yolk sac, at least in the early stages. Antibodies may also be transferred to the embryo by this route (Brambell, Hemming, Henderson, Parry & Rowlands, 1950).

The cytoplasmic basophilia of the trophoblastic epithelium and its rich alkaline phosphatase content are apparently fundamental histochemical properties of the mammalian placenta (Wislocki *et al.* 1946). The former is probably due to the presence of ribonucleoprotein, since it is abolished by ribonuclease; the trophoblast may, therefore, be an important site of absorption, storage or synthesis of this material. The significance of the alkaline phosphatase in the placenta is not clear and has been discussed in the rat by Pritchard (1947) and in the guinea-pig by Hard (1946). It may be concerned in the placental transport or synthesis of fats, carbohydrates and nucleoproteins which may be dependent on a phosphorylation process. There is evidence that the passage of simple hexoses, such as glucose and fructose, across the placental membrane may be an 'active process' and not one of simple diffusion (Huggett, Warren & Warren, 1951; Karvönen & Riih , 1954; Davies, 1955). It must be remembered, however, that almost all the known groups of enzymes have been demonstrated chemically in the mammalian placenta (Page & Glendenning, 1955), and it is possible that undue emphasis has been placed on the alkaline phosphatase group since simple methods are available for their histochemical identification.

Large amounts of glycogen are found histochemically as early as the 12th or 13th day in the rabbit placenta; it is probable that it can be demonstrated much earlier. Chipman (1902) and Maximow (1900) stated that it was maximal from the 12th to the 16th day. Lockhead & Cramer (1908), using chemical methods, observed that glycogen was abundant from the 18th to the 22nd day and was still present in the 'zone of separation' at term. Several authorities (Lockhead & Cramer, 1908; Huggett, 1929) have commented on the remarkable stability of the placental glycogen in the rabbit under experimental conditions which deplete the foetal liver and body glycogen. It is perhaps noteworthy that the placental glycogen is more readily preserved by routine histological fixatives, such as Bouin's or Zenker's fluid, than is the liver glycogen.

The metachromatic reaction of the decidual ground substance, together with reaction with the PAS and aldehyde fuchsin stain, suggest the presence of an acid mucopolysaccharide. Similar material has been demonstrated in relation to the basal plate of the human placenta at term (Wislocki, 1951) and in the stroma of the human uterus in the menstrual cycle and in early pregnancy (Halmi & Davies, 1954). It may represent a highly polymerized material which may be the ultimate barrier to the continued invasion of the trophoblast.

The nature of the placental fat is not clear. It has been considered to be partly phospholipid (Hard, 1946) and, by others (Dempsey & Wislocki, 1944), histochemical evidence has been presented in favour of its steroid nature, at least in the human placenta where it may represent the site of storage or synthesis of the steroid hormones. Gomori (1952) has criticized the chemical basis for the identification of the fat as steroid. Beyond confirming the presence of osmiophilic material in the trophoblast at all stages in the rabbit, the studies presented in this paper add nothing to the understanding of this problem.

In conclusion, the concept of the late rabbit placenta as haemo-endothelial rather than haemochorial has not been substantiated. A similar conclusion was reached in the rodents by Wislocki *et al.* (1946), by Hard (1946) in the guinea-pig, and by Pritchard (1947) in the rat. Impressive evidence against the haemo-endothelial concept has been derived from studies by the electron microscope in the rat and rabbit by Wislocki & Dempsey (1955).

SUMMARY

1. Histochemical studies of the rabbit placenta from about the 12th day to term have shown the following features:

(a) The trophoblastic epithelium contains basophilic material, probably ribonucleoprotein, since it is removed by ribonuclease, alkaline phosphatase and fat, but no glycogen at any stage.

(b) The ground substance of the decidual stroma is metachromatic, PAS and aldehyde fuchsin positive. These reactions may indicate the presence of an acid mucopolysaccharide. The uterine stroma is also rich in alkaline phosphatase.

(c) The uterine epithelium is strongly basophilic (ribonucleoprotein), has a PAS-positive brush border, contains PAS-positive droplets (probably glycoprotein) but no glycogen or alkaline phosphatase.

(d) The yolk-sac epithelium is columnar, has a PAS-positive brush border in which a variable reaction for alkaline phosphatase may be demonstrated in the middle third of gestation; the cells contain many PAS-positive granules, which are also acidophilic, stain with iron haematoxylin and are, in general, Gram-positive. The droplets may represent glycoprotein absorbed from the uterine lumen. The yolk-sac epithelium also contains basophilic material (ribonucleoprotein), especially in the middle third of gestation. It also contains fat but no glycogen.

(e) The possible significance of these histochemical findings is discussed with special reference to the problems of the placental transport of carbohydrates and of the transport, storage or synthesis of fat and protein.

REFERENCES

- AMOROSO, E. C. (1952). Placentation. In Marshall's *Physiology of Reproduction*, vol. 2. New York, London: Longmans, Green and Co.
- BRAMBELL, F. W. R., HEMMING, W. A., HENDERSON, M., PARRY, H. J. & ROWLANDS, W. T. (1950). The route of antibodies passing from the maternal to the foetal circulation in rabbits. *Proc. Roy. Soc. B*, **136**, 131-294.
- CHIPMAN, W. (1902). Observations on the rabbit with special reference to the presence of glycogen, fat and iron. *Stud. R. Victoria Hosp., Montreal*, vol. **1**, Gynec. 1, pp. 1-261.

- DAVIES, J. (1954). Cytological evidence of protein absorption in fetal and adult mammalian kidneys. *Amer. J. Anat.* **94**, 45-72.
- DAVIES, J. (1955). Permeability of the rabbit placenta to glucose and fructose. *Amer. J. Physiol.* (in the Press).
- DEMPSEY, E. W. & WISLOCKI, G. B. (1944). Observations on some reactions of the human placenta with special reference to the significance of the lipoids, glycogen and iron. *Endocrinology*, **35**, 409-429.
- DUVAL, M. M. (1892). *Le placenta des rongeurs: lapin*. Paris: Félix Alcan.
- GOMORI, G. (1939). Microtechnical demonstration of phosphatases in tissue sections. *Proc. Soc. Exp. Biol.* **42**, 23-26.
- GOMORI, G. (1950a). Aldehyde fuchsin: a new stain for elastic tissue. *Amer. J. clin. Path.* **20**, 665-666.
- GOMORI, G. (1950b). A rapid one-step trichrome stain. *Amer. J. clin. Path.* **20**, 661-665.
- GOMORI, G. (1952). *Microscopic Histochemistry*. Chicago: University of Chicago Press.
- HALMI, N. S. & DAVIES, J. (1953). Comparison of aldehyde fuchsin, metachromasia, and periodic acid-Schiff reactivity of various tissues. *J. Hist. Cytoch.* **1**, 447-459.
- HARD, W. L. (1946). A histochemical and quantitative study of phosphatases in the placenta and fetal membranes of the guinea pig. *Amer. J. Anat.* **78**, 47-67.
- HUGGETT, A. St G. (1929). Maternal control of the foetal glycogen. *J. Physiol.* **67**, 360-371.
- HUGGETT, A. St G., WARREN, F. L. & WARREN, N. V. (1951). The origin of the blood fructose in the foetal sheep. *J. Physiol.* **113**, 258-275.
- KARVÖNEN, M. J. & RAIHÄ, N. (1954). Permeability of the guinea pig placenta to glucose and fructose. *Acta physiol. scand.* **31**, 194-202.
- LILLIE, R. D. (1953). *Histopathologic Technique and Practical Histochemistry*. Toronto: Blakiston.
- LOCKHEAD, J. & CRAMER, W. (1908). The glycogenic changes in the placenta and foetus of the pregnant rabbit. *Proc. Roy. Soc. B*, **80**, 263-284.
- MAXIMOW, A. (1900). Die erste Entwicklung der Kaninchen Placenta. *Arch. mikr. Anat.* **56**, 699-740.
- MOSSMAN, H. W. (1937). Comparative morphogenesis of the fetal membranes. *Contr. Embryol. Carneg. Instn*, **26**, 129-246.
- PAGE, E. W. & GLENDENNING, M. B. (1955). *Transactions First Conference on Gestation*, pp. 225-229. Josiah Macy, Jr. Foundation.
- PRITCHARD, J. J. (1947). The distribution of alkaline phosphatase in the pregnant uterus of the rat. *J. Anat., Lond.*, **81**, 352-366.
- WISLOCKI, G. B. (1951). The histology and cytochemistry of the basal plate and septa placentae of the normal human placenta delivered at term. *Anat. Rec.* **109**, 359.
- WISLOCKI, G. B., DEANE, H. W. & DEMPSEY, E. W. (1946). The histochemistry of the rodent's placenta. *Amer. J. Anat.* **78**, 281-345.
- WISLOCKI, G. B. & DEMPSEY, E. W. (1955). *Transactions First Conference on Gestation*, pp. 178 et seq. Josiah Macy, Jr. Foundation.

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Transverse section of the uterine capsule of a rabbit at about the 13th day and containing an embryo of 7 mm. Glycogen is abundant in the decidual tissues of the subplacental and paraplacental regions and also in isolated plaques at several points around the uterine wall. The trophoblast contains no glycogen. The amniotic sac (A) is small. There is a large amount of yolk material (Y) between the atrophic outer wall (O) and the highly differentiated inner (I) wall of the yolk sac; it is faintly PAS-positive. Bouin. PAS method. $\times 3\frac{1}{2}$.
- Fig. 2. Details of the distribution of glycogen in the subplacental area of the same placenta as in fig. 1. The decidual cells, rich in glycogen, are grouped around the large vascular sinuses. In the deeper part of the subplacenta, adjoining the uterine muscle (to the right), the glycogen containing cells form a continuous sheet or 'zone of separation'. Bouin. PAS. $\times 30$.
- Fig. 3. Details of the decidual tissues of the subplacental area at about 17 days showing the strong metachromasia of the ground substance. The endothelial giant cells of the uterine sinuses are basophilic. Bouin. Toluidine blue. $\times 70$.

Fig. 4. Subplacenta area of rabbit at about the 17th day showing the intense alkaline phosphatase activity of the decidual stroma. The endothelial giant cells also show enzymic activity. Absolute alcohol. Gomori method (sodium glycerophosphate, one hour). $\times 70$.

PLATE 2

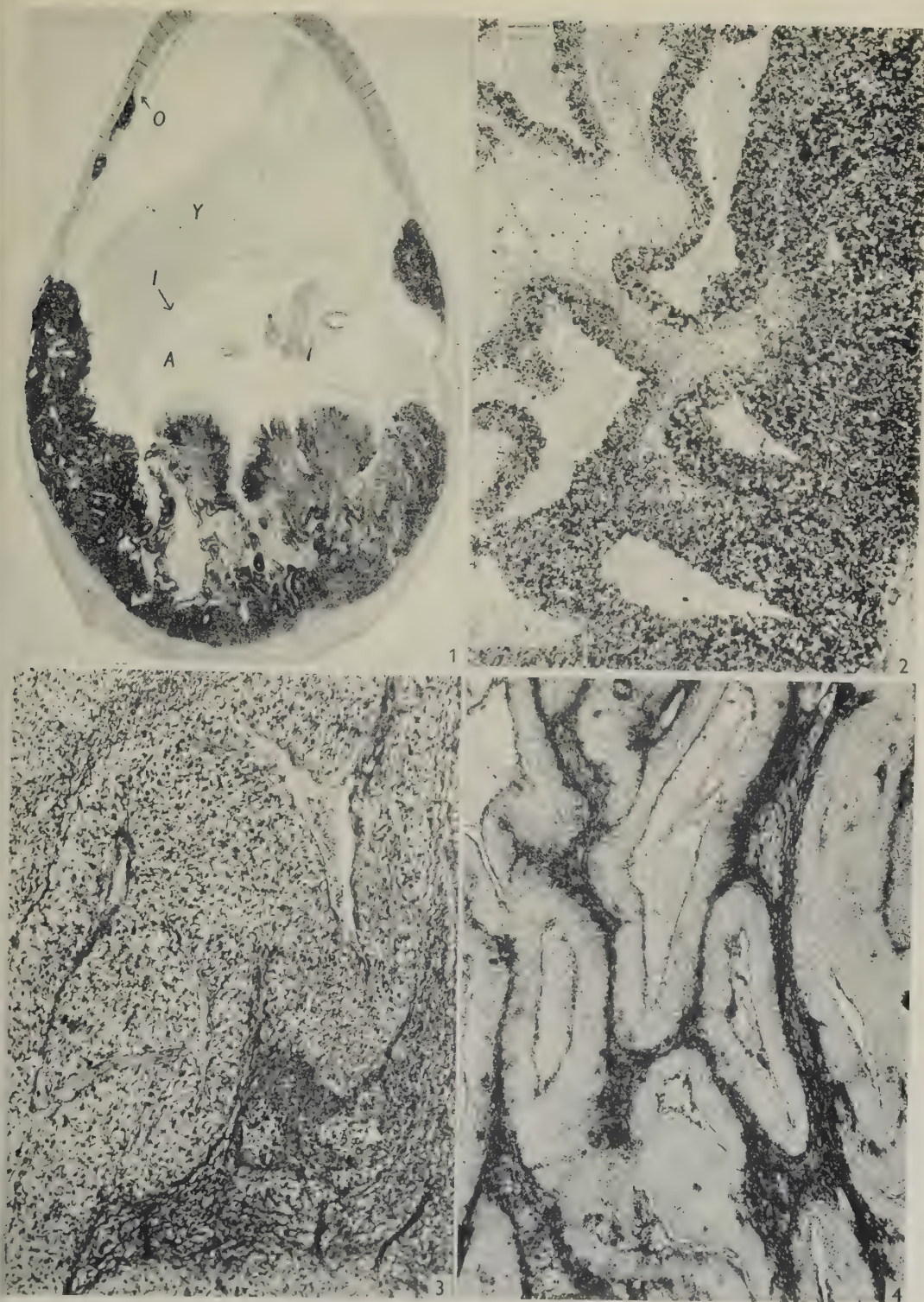
- Fig. 5. The 'zone of separation' from a placenta at term. The PAS positivity of the decidual ground substance is illustrated (see also fig. 3). Between the decidual cells and the trophoblast (to the right) is a thick zone of necrotic material which is also PAS-positive. Bouin. PAS after saliva. $\times 35$.
- Fig. 6. The uterine epithelium from a rabbit at about the 17th day of gestation showing the 'brush border', the syncytial transformation of a part of the epithelium and the large numbers of PAS-positive droplets contained within these cells. The uterine milk is also PAS-positive. Bouin. PAS after saliva. $\times 300$.
- Fig. 7. Portion of the uterine wall at about the 17th day showing the intense stromal reaction for alkaline phosphatase. The epithelium shows no reaction. Absolute alcohol. Gomori method as in fig. 4. $\times 80$.
- Fig. 8. The trophoblast in the region of the intercotyledonary groove at 17 days illustrating the strong reaction for alkaline phosphatase. A maternal vessel is shown at the top of the figure and may be seen emptying into the trophoblastic columns. The thin-walled allantois may also be seen extending deeply into the sulcus between the cotyledons. Gomori technique, as in fig. 4, time 30 min. $\times 30$.
- Fig. 9. Section through the intercotyledonary sulcus at the 17th day showing the cuboidal allantoic epithelium and the tendency to form gland-like spaces. Bouin. Haematoxylin and trichrome. $\times 140$.

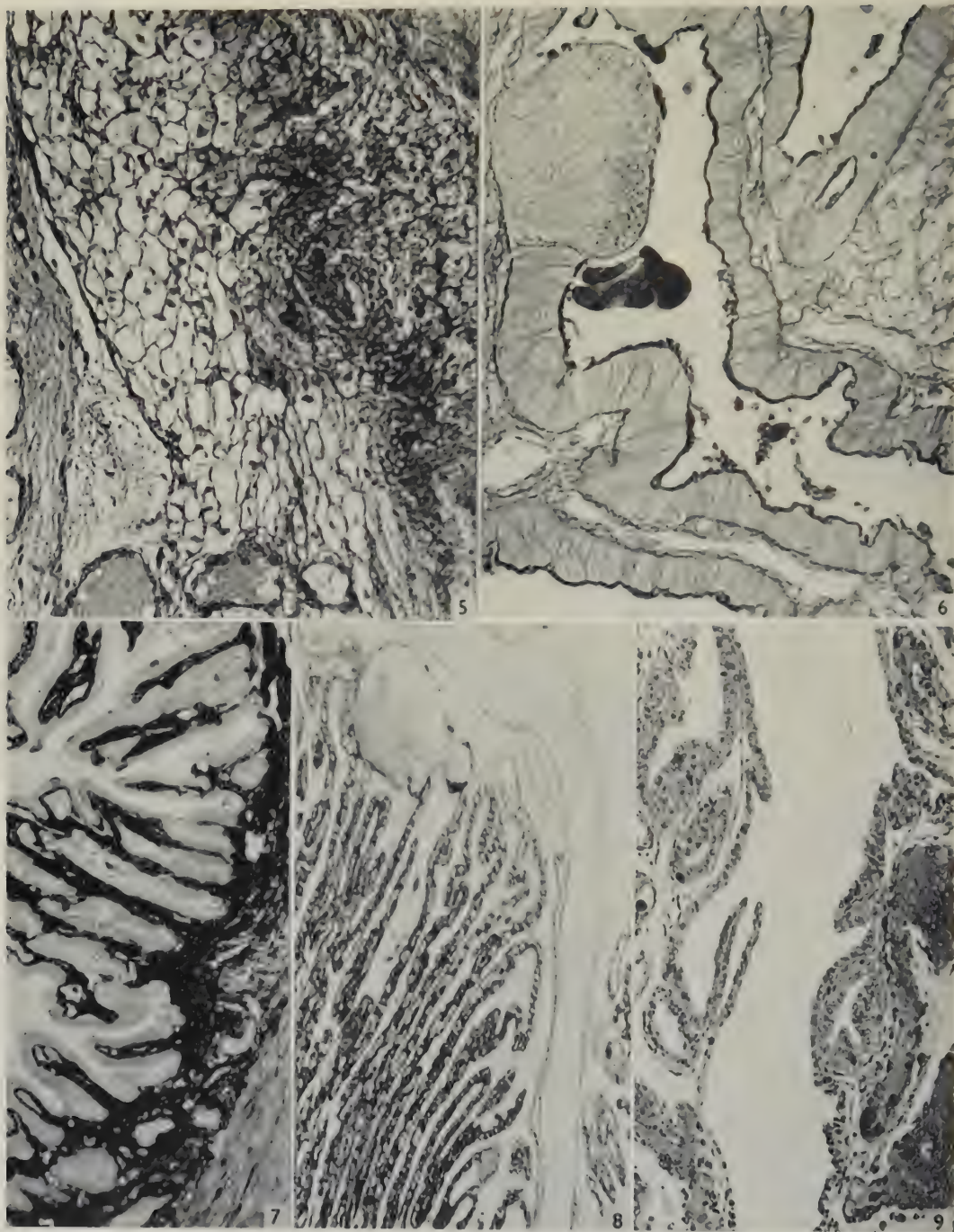
PLATE 3

- Fig. 10. Section of the allanto-placenta of a rabbit of 22 days showing the intense cytoplasmic basophilia of the trophoblastic epithelium. A small nest of decidual cells is seen at the bottom of the figure; these cells are not basophilic. Bouin. Toluidine blue. $\times 120$.
- Fig. 11. Trophoblastic epithelium at the 17th day containing many discrete droplets of fat. Flemming, paraffin sectioning. $\times 200$.
- Fig. 12. Details of the placental labyrinth at term. The basement membrane of the foetal vessels between the trophoblastic 'tubes' (Duval) is strongly PAS-positive. There is almost no foetal mesenchyme interposed between the trophoblast and the basement membrane of the foetal vessels. Though attenuated the trophoblastic envelope is present at all points. Bouin. PAS after saliva. $\times 400$.

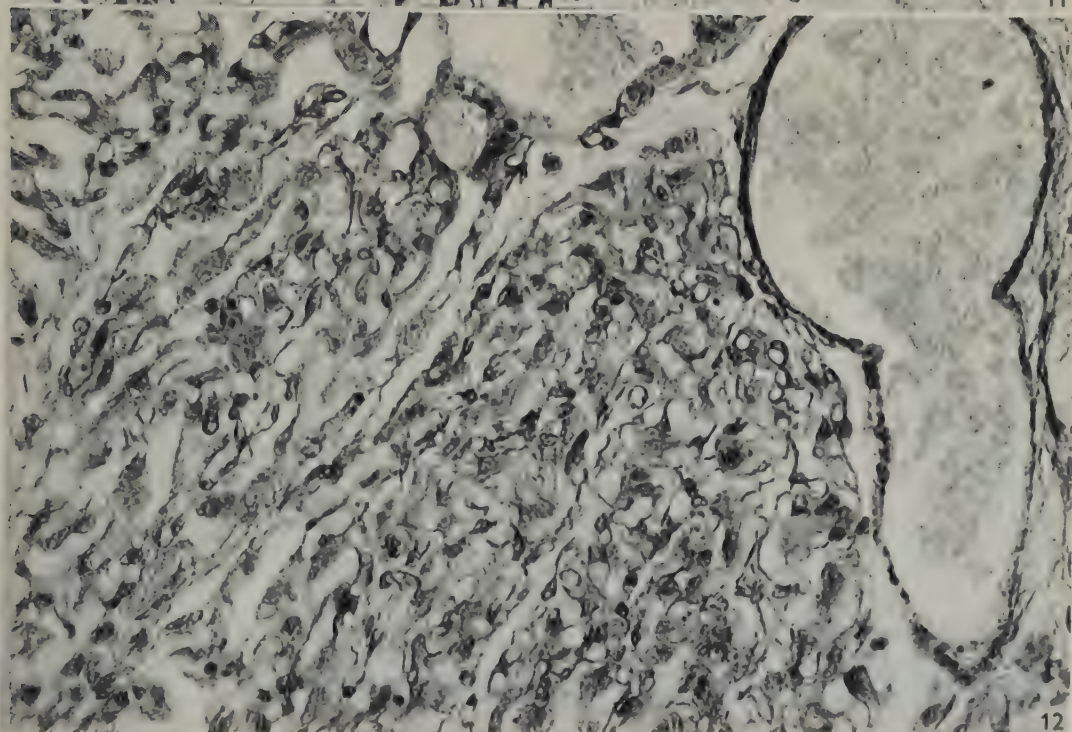
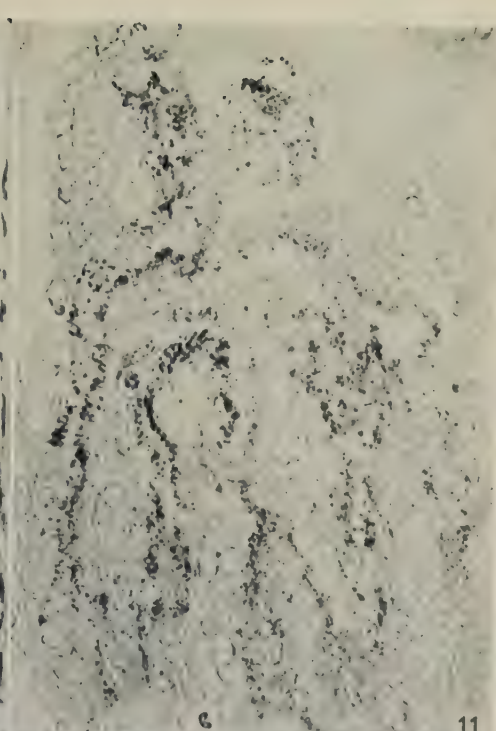
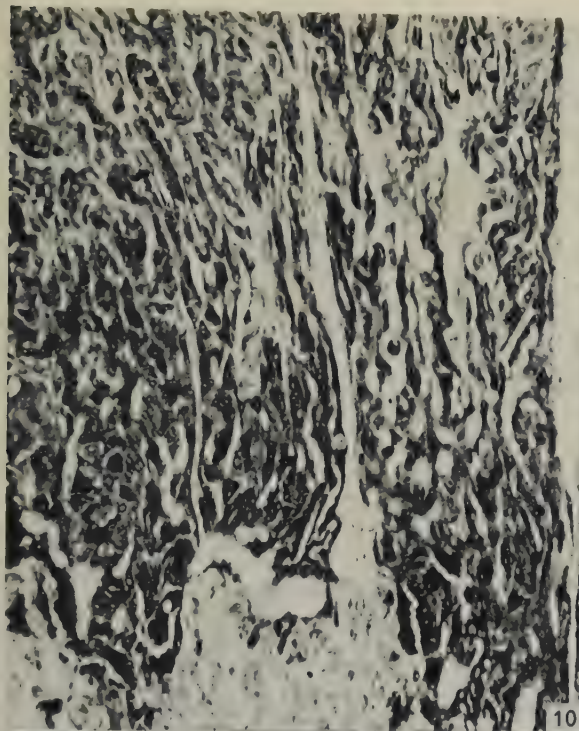
PLATE 4

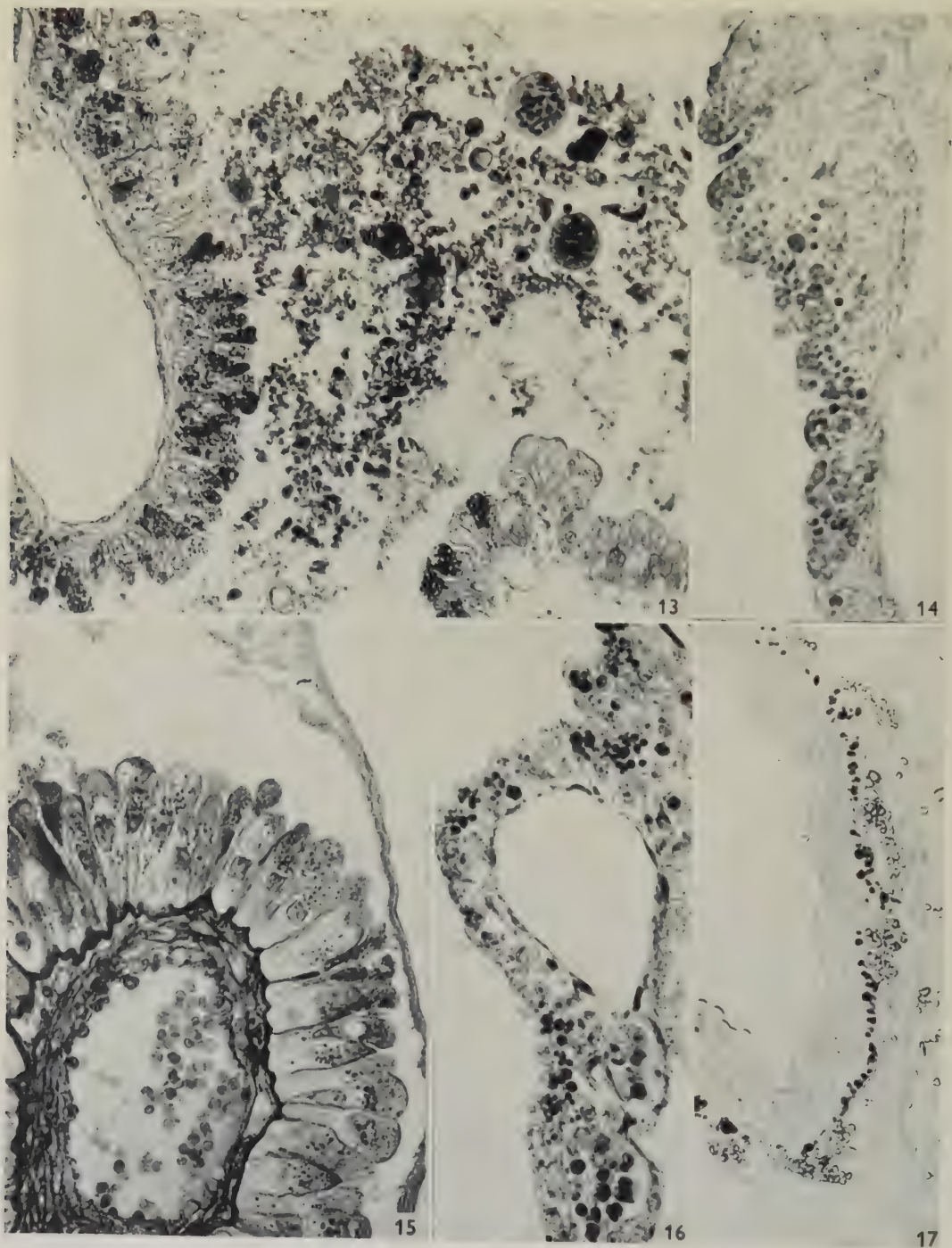
- Fig. 13. Yolk sac and adjoining uterine lumen from a rabbit at the 15th day of gestation. The columnar cells of the yolk sac contain many droplets which have similar staining properties to those of the 'uterine milk'. The latter also contains many multinucleate giant cells of trophoblastic origin and much acidophilic cellular debris. Bouin. Iron haematoxylin and trichrome. $\times 150$.
- Fig. 14. Yolk-sac epithelium at the 13th day showing the PAS-positive staining of the intracellular droplets. Bouin. PAS after saliva. $\times 400$.
- Fig. 15. Yolk sac at term illustrating the villous character of the wall and the character of the columnar epithelium which contains PAS-positive droplets but fewer in number than in earlier stages (figs. 13 & 14). The basement membrane is thickened, as is the subepithelial stroma, and both are PAS-positive. Bouin. PAS after saliva. $\times 350$.
- Fig. 16. Epithelium of yolk sac at the 13th day indicating the affinity of the droplets for iron haematoxylin. Bouin. Iron haematoxylin. $\times 350$.
- Fig. 17. Yolk sac at the 15th day showing the discrete droplets of fat which are basally located in the cells. Flemming. Paraffin sectioning. $\times 400$.





DAVIES—HISTOCHEMISTRY OF THE RABBIT PLACENTA





DAVIES—HISTOCHEMISTRY OF THE RABBIT PLACENTA

THE DUCTUS VENOSUS OF THE PIG

By A. D. DICKSON

Department of Anatomy, University of Cambridge

INTRODUCTION

The ductus venosus, which appears at about the 5 mm. stage in a variety of mammalian species, brings into communication the advehent and revehent sides of the hepatic circulation. In its definitive condition, the ductus venosus takes origin from the left end of the sinus intermedius (portal sinus), opposite to the opening into that vessel of the umbilical vein, and terminates in the dorsal aspect of the left hepatic vein, close to the entrance of the latter into the posterior/inferior vena cava. Thus the connexions of the ductus are such that it must, while it remains patent, act as a by-pass which enables at least some of the oxygenated blood returning from the placenta in the umbilical vein to short-circuit the complex sinusoidal circulation of the liver and to pass easily and rapidly to the heart. It has long been supposed that the major portion of the umbilical venous blood does, in fact, traverse the ductus venosus, and that it is the requirement by the brain and head of large quantities of richly oxygenated blood which dictates this course. The first doubts as to the correctness of this generally accepted view were expressed by Barclay, Franklin & Prichard (1944), who had applied the technique of cine-radiography to the intact foetal sheep. They expressed the opinion that the amount of blood traversing the ductus is small, being about one-ninth of that entering the foetus in the umbilical vein. This estimate was based on the ratio of the cross-sectional areas of the vessels, somewhat unsoundly, it must be said, for the proportions of the umbilical venous blood traversing the alternative routes, namely, the ductus venosus and the hepatic sinusoids, will be related to the resistances to flow in these routes. However, these authors sowed the seeds of doubt, and the discovery of a sphincter at the origin of the ductus (Barron 1942) suggested the idea that the function of the ductus venosus is not that of a simple by-pass, but rather of a valvular mechanism. Barclay *et al.* (1944) considered that the sphincter of the ductus could control the amount of blood traversing the liver sinusoids, but that its main function was to occlude the ductus at birth. Reynolds (1951) postulated that the function of the ductus with its sphincter is to maintain the pressure in the umbilical vein, or veins, thus preventing collapse. He also showed that pressure changes of the order of 1 cm. of water are transmitted to the umbilical veins by the umbilical arteries in the cord, and he suggested that, in addition to keeping the veins distended, the ductus sphincter, by its contraction or relaxation, acts as a valve which evens out these pressure changes.

It is not yet, however, generally accepted that it is the function of the ductus venosus to control intra-vascular pressures. Indeed, the old concept of the ductus as a simple by-pass still has currency. For example, Dawes, Mott & Widdicombe (1954), who made a quantitative study of the foetal circulation by comparing the oxygen contents of samples of blood taken from a number of vessels of a living sheep

foetus, make the assumption that the umbilical venous blood gives off no oxygen to the liver, an assumption which seems to indicate their belief in the passage of the major proportion of the umbilical blood through the ductus venosus to the posterior vena cava.

In view of these conflicting opinions concerning the significance of the ductus venosus, it is of great interest to note that, according to Klages (1931) and Barclay *et al.* (1944), the ductus venosus of the pig disappears during intra-uterine life. Beyond the bare fact of this disappearance, these authors do not go. The fate of the vessel in this species has, therefore, been closely studied, for the vascular arrangements in an animal which can dispense with its ductus may well be expected to throw light on the function of the vessel in those species in which it persists until birth.

MATERIAL AND METHODS

The material utilized in this investigation consists of 14, 18, 20 and 24 mm. pig embryos, 47, 100, 170 and 210 mm. pig foetuses, a 1-day-old pig of 270 mm. and a 14-day-old pig of 300 mm. Each measurement is that of the crown-rump length of a formalin-fixed specimen. Serial sections, cut in the transverse plane at 10 μ , were made of the whole specimen or of the liver only, according to size, except in the cases of the 100 and 170 mm. foetuses and the 1-day-old pig, from which the livers were removed and cut transversely by hand into thin slices for macroscopic examination. The serial sections of the 14, 18, 20 and 24 mm. embryos and of the 14-day-old pig were stained with haematoxylin and eosin. In the 47 mm. foetus, one slide in three was stained with Masson's trichrome stain, the remainder being stained with haematoxylin and eosin. The sections of the 210 mm. specimen were stained with haematoxylin and eosin, Masson's trichrome stain and van Gieson's stain, one slide out of three being treated with each stain.

OBSERVATIONS

14 mm. pig embryo

The early development of the ductus venosus of the pig has not been described as fully as has that of man by such workers as Schneider (1937) and Huzly (1942). In its disposition and its relations to the major hepatic vessels, however, the ductus of the embryonic pig is essentially similar to that of man. At the 14 mm. stage it is found to take origin from the left, or ventral, end of the sinus intermedius. This sinus lies slightly obliquely on the deeply concave visceral surface of the liver, and its left end is on a plane ventral to its right. The umbilical and portal veins terminate in the left and right ends, respectively, of the sinus. The ductus venosus runs cranially in the mid-line, lying near the attachment of the lesser omentum to the dorsal surface of the liver, and terminates in the dorsal aspect of the left hepatic vein close to the entrance of the latter into the posterior vena cava. This last-named vessel receives the right hepatic vein at the same cranio-caudal level. Thus, at the 14 mm. stage, the ductus venosus of the pig has the position and connexions typical of the vessel in a wide range of mammalian species.

18 mm. pig embryo

The ductus venosus still arises from the left end of the sinus intermedius and terminates in the dorsal aspect of the left hepatic vein. Another vessel arises from the sinus intermedius immediately to the right of the origin of the ductus venosus (Pl. 1, fig. 1). This vessel courses dorsally, cranially and to the right, supplying the liver substance lying between the ductus venosus and the posterior vena cava. This portion of the liver, which used to be called the Spigelian lobe, was named the omental lobe by Rex (1888). The vessel supplying it he termed the ramus omentalis.

The ductus venosus, as it passes cranially to its termination, has numerous small communications with the neighbouring sinusoids. There is also, and this is worthy of note, a larger vessel connecting it with the ramus omentalis.

20 mm. pig embryo

The origin of the ductus venosus is now slightly constricted. The ductus and the ramus omentalis arise close together from the left, or ventral, end of the sinus intermedius. They run cranially on slightly diverging courses. The ramus omentalis is much larger than appears to be required by the number and the small size of the branches which it gives to the omental lobe. Its size would seem to be accounted for by the considerable number of anastomotic channels which it sends to the ductus venosus. Two of these channels, which are separated by septa of hepatic epithelium, can be seen in Pl. 1, fig. 2. The hepatic epithelium of the septa is separated from the blood in the anastomotic channels only by a minute amount of connective tissue and the endothelium of the channel wall.

The ductus venosus receives blood from the ramus omentalis through the sinusoids of the omental lobe, in addition to that which comes through the anastomotic channels mentioned above. These sinusoids enter the ductus cranial to the point at which the ramus omentalis breaks up into its branches to the ductus venosus and the omental lobe.

24 mm. pig embryo

In this specimen the origin of the ductus venosus from the left end of the sinus intermedius is markedly constricted. The ramus omentalis is large and sends numerous anastomotic channels to the ductus venosus. The amount of hepatic substance between the ramus omentalis and the ductus venosus is greater than in the 20 mm. specimen, as is the angle of divergence between the two vessels. Thus the passage of the anastomotic channels through the liver is longer. Some of the channels are, as in the 20 mm. embryo, quite large (Pl. 1, fig. 3).

47 mm. pig foetus

The origin of the ductus venosus from the sinus intermedius, which was constricted in the 24 mm. embryo, is now completely obliterated. The ductus, which is thus blind caudally, remains patent, however, throughout most of its length and still terminates in the dorsal surface of the left hepatic vein. At this stage the ductus receives all its blood from the anastomotic channels, which now enter it from both

sides and not only from the left side as in smaller specimens. On the right side the channels are derived from the ramus omentalis, as before, and on the left side from a branch of the ramus angularis, which is the vessel of supply to the left lateral lobe of the liver. The ramus omentalis and this branch of the ramus angularis run dorsally in pincer fashion around the ductus a short distance cranial to its blind caudal extremity. They give origin to anastomotic channels which pass through the liver substance to the ductus venosus (Pl. 1, fig. 4). Further, both the ramus omentalis and the branch of the ramus angularis send vessels cranially. These vessels lie at intervals around the periphery of a cone, in the long axis of which is the persisting cranial portion of the ductus venosus. They send numerous anastomotic channels through the liver substance to the ductus venosus, becoming gradually smaller as they do so. They terminate, caudal to the opening of the ductus venosus into the left hepatic vein, as the final anastomotic channels entering the ductus.

In this specimen, the course of the anastomotic channels through the liver is longer than in the 24 mm. embryo, for the supplying vessels are farther from the ductus venosus (possibly due to growth of the liver). The channels are smaller, absolutely as well as relatively, than those of the 24 mm. specimen. The thinness of the channel walls, and the intimacy of the relationship between the contained blood and the hepatic epithelium, remain unaltered.

100 mm. pig foetus

In this specimen, the liver of which was cut into thin slices by hand, a large number of vessels lie clumped together in the liver substance close to the attachment of the lesser omentum (as indicated by an arrow in Pl. 1, fig. 5). This clump of vessels represents the plexus which is in part replacing the ductus venosus, the cranial portion of which is still present and which drains the plexus into the left hepatic vein.

170 mm. pig foetus

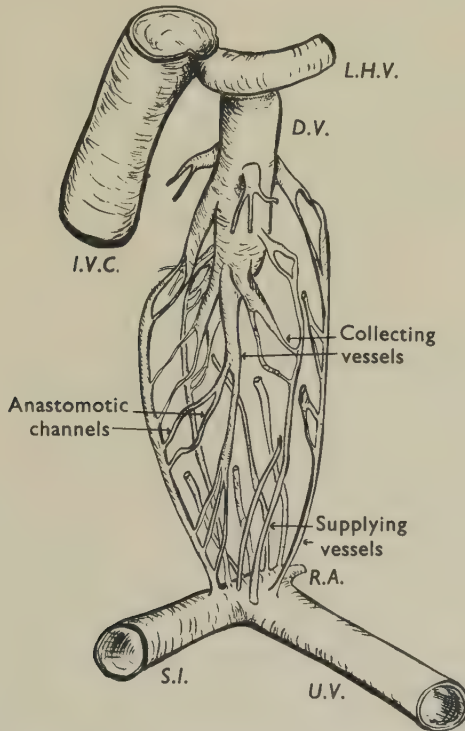
The liver of this specimen was also cut into thin slices by hand. A plexus of small vessels, which originate from the left end of the sinus intermedius and terminate in the persisting cranial portion of the ductus venosus, lies close to the line of the attachment of the lesser omentum. The detailed arrangements of the plexus cannot be made out by naked-eye examination.

210 mm. pig foetus

The supplying vessels (Text-fig. 1) now arise from the left end of the sinus intermedius and from the origin of the ramus angularis, the ramus omentalis being no longer recognizable as an entity. They pass cranially, lying at intervals around the periphery of a cone. They despatch blood centripetally into the anastomotic channels, and through them into large collecting vessels, all of which drain into the persisting cranial portion of the ductus venosus and thence into the left hepatic vein. The anastomotic channels are both longer and narrower than in the earlier specimens, while the walls of the channels remain as thin as before, consisting of endothelium and an extremely fine layer of collagenous connective tissue (Pl. 2, fig. 6). The blood in the channels, therefore, continues to come into intimate relationship with the

hepatic epithelial cells, and now over a greater length than before, owing to the increased length of the channels. The collecting vessels have, like the supplying vessels, several layers of collagen outside their endothelial linings.

There are scattered among the vessels of the plexus masses of hepatic epithelium of considerable size (Pl. 2, fig. 7). Each of these masses contains a sinusoidal circulation, the blood in which comes from the supplying vessels and drains into the collecting vessels. This blood comes into more intimate relationship with hepatic cells



Text-fig. 1. Diagram to illustrate the arrangement, in a 210 mm. pig foetus, of the vessels which have replaced the caudal portion of the ductus venosus. *D.V.* ductus venosus; *I.V.C.* posterior vena cava; *L.H.V.* left hepatic vein; *R.A.* ramus angularis; *S.I.* sinus intermedius; *U.V.* umbilical vein.

than does the blood which traverses the anastomotic channels. The fact that these masses are supplied and drained by vessels of the plexus indicates that they develop from the very much smaller amounts of hepatic tissue which lay between the supplying vessels and the ductus venosus in smaller specimens.

1-day-old pig

In the post-natal specimen, as in the 210 mm. foetus, the supplying vessels run cranially from the left, or ventral, end of the sinus intermedius, lying at intervals around the periphery of a cone (Pl. 2, fig. 8). These supplying vessels are separated from the collecting vessels by a considerable amount of hepatic epithelium. A

striking feature at this stage is the relatively small number of vessels to be seen in a cross-section of the ductus region. Such as are visible are isolated vessels of the larger sizes, that is, supplying or collecting vessels. It would appear that by the increase of the liver tissue the anastomotic channels have been converted into sinusoids.

14-day-old pig

This specimen, which was sectioned serially and examined microscopically, confirms the impression obtained from the study of the liver of the 1-day-old pig that the supplying vessels are separated from the collecting vessels by considerable amounts of hepatic epithelium, the blood passing from one set of vessels to the other through sinusoids rather than through the larger anastomotic channels of earlier stages (Pl. 2, fig. 9). The supplying vessels, the disposition of which is similar to that of the 210 mm. and 1-day-old specimens, now have considerably thicker walls than have the collecting vessels. The latter are radicles of the persisting cranial portion of the ductus venosus which still retains its opening into the left hepatic vein.

DISCUSSION

The alterations described above, which affect the ductus venosus of the pig, are, as far as is known at present, quite peculiar to this mammal, though Barclay *et al.* (1944) state that the ductus of the horse foetus disappears, and it may be that similar changes will be found to occur in that species.

Up to about the 18 mm. stage, the ductus venosus of the pig is similar in its course and relations to that of man and such other mammals as the sheep, the goat and the rat. Alterations commence to occur at about the 18 mm. stage which transform the ductus venosus into a hepatic vein, which collects blood from its own territory of the liver and is a radicle of the left hepatic vein. It is worthy of note that at no stage in the pig is there smooth muscle in the wall of the ductus venosus, or of its associated vessels, so that there exists nothing comparable to the sphincter of the ductus of other mammals.

The fundamental alterations in the pig are the obliteration of the caudal end of the ductus, which occurs between the 24 and 47 mm. stages, and the establishment of anastomotic channels between the persisting cranial portion of the ductus and the vessels which supply blood to the neighbouring parts of the liver. After the anastomotic channels have been formed and the caudal end of the ductus has been obliterated, the ensuing changes, though exceedingly complex in their details, appear to be directed towards one end, namely, the bringing of the blood passing in the anastomotic channels to the ductus venosus into ever more intimate relationship with the hepatic epithelial cells. These channels, from their first appearance, have thin walls, the barrier separating the blood in them from the hepatic cells consisting, at most, of endothelium and one layer of collagenous connective tissue. The channels, however, are at first wide, so that the contact between the blood in them and the surrounding hepatic epithelium cannot be regarded as intimate. As development proceeds the channels become longer and narrower, making the contact more intimate. The appearances suggest that ultimately the anastomotic channels become sinusoids by their increasing complexity and by the multiplication of the

hepatic trabeculae pervading the plexus. The circulation is thus entirely sinusoidal in the 14-day post-natal specimen, as it probably is also in the 1-day-old pig. It may be, and probably is, sinusoidal even before birth. Moreover, the structure of the part of the liver of the 14-day-old pig which is drained by the persisting cranial portion of the ductus venosus, is indistinguishable from the structure of the liver elsewhere. The presence of this cranial portion of the ductus venosus in a post-natal specimen suggests that it will persist in the full-grown pig as a radicle of the left hepatic vein draining its own territory of the liver.

The present investigation, while it has revealed the fate of the ductus venosus of the pig, has not demonstrated any mechanism compensating for the absence of the ductus, that is, no other short-circuit or by-pass takes its place. Therefore, once the alterations described are well under way, all the blood returning from the placenta must pass through the liver sinusoids, and must, moreover, come into intimate relationship with the liver cells. This suggests that the re-routing of the placental blood may be dictated by metabolic requirements. If the metabolism of the pig requires that the whole of the blood returning laden with nutriment and oxygen from the placenta act upon, or be acted upon by, the liver before release to the general circulation, then it seems not unreasonable to suppose that the metabolism of those species in which the ductus venosus persists until birth will require a large proportion of the placental blood to pass through the liver sinusoids. If this is the case, then it may be argued that the ductus venosus in these species is not a simple by-pass, but is, more probably, a valvular pressure-controlling mechanism. In short, then, the disappearance of the ductus venosus as a continuous channel in the pig could be interpreted as a verification, admittedly somewhat oblique, of the hypothesis of the valvular function of the ductus venosus.

It is now necessary to examine this hypothesis more closely. If the ductus venosus acts as a valve during intra-uterine life, then its sphincter must develop at an early stage. Chacko & Reynolds (1953) have shown that it does in fact develop during the embryonic period in man (and I have found it to be present at the 11 mm. stage in man, at the 15 mm. stage in the goat and in the 16-day rat embryo). The structural basis of the valvular mechanism being present, then, in the form of the smooth muscle of the sphincter, during the latter part of embryonic and the whole of foetal life, what does the valve control? Reynolds (1951) has shown, as mentioned earlier, that pressure changes are, in the sheep, transmitted to the umbilical veins from the umbilical arteries in the cord. It is likely also that contractions of the uterus cause pressure changes in the umbilical veins. Reynolds has suggested that, through the relaxation or contraction of its sphincter, the ductus protects the liver by smoothing out these fluctuations in the pressure of the blood in the umbilical vein.

While it is probably quite true that the umbilical vein pressure is smoothed by the sphincter of the ductus, further consideration of the anatomical relationships of the hepatic circulation indicates that it is not the liver which is in most need of protection from pressure fluctuations. Paltauf (1888), many years before the sphincter of the ductus venosus was discovered, considered that the function of the ductus is to keep the pressures in the umbilical and portal veins in equilibrium. This suggestion was forgotten, and the point has ever since been missed that if two vessels terminate in opposite ends of one channel, and if the pressure in one or both vessels fluctuates,

then there must be a mechanism for keeping the pressures in these vessels in equilibrium. The resistance in the sinusoids being, as far as is known, constant in all parts of the liver, an increase of pressure in the umbilical vein would tend to cause a reversal of flow in the portal vein, were there no controlling mechanism such as the ductus venosus appears fitted to provide. Barclay *et al.* (1944) showed that, in the sheep, the umbilical vein blood passes along the connecting channel, the sinus intermedius, to the right end, but does not enter the hepatic territory of the portal vein. It is now suggested that it is the function of the ductus venosus to maintain this condition constant, the sphincter relaxing when the pressure in the umbilical vein tends to rise in relation to the pressure in the portal vein, and contracting in the opposite circumstances. It follows, consequentially, that the amount of blood flowing through the ductus venosus at any moment is a function of the relationship between the pressures in the umbilical and portal veins at that moment.

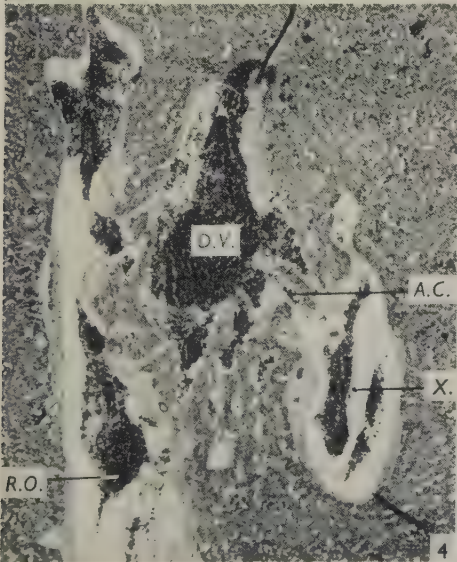
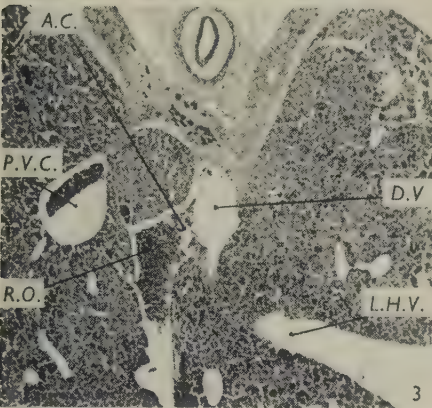
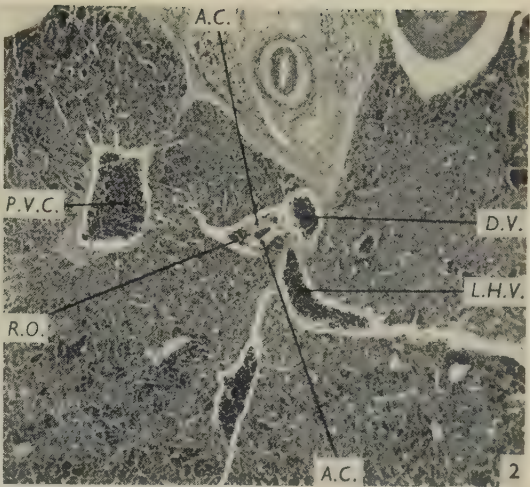
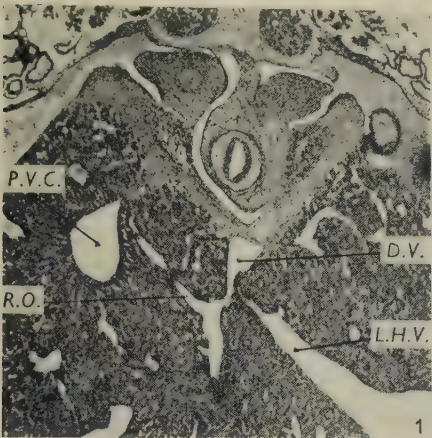
This hypothesis concerning the function of the ductus venosus, while speculative and tentative, fits all the facts known about the hepatic circulation of the living foetus. If it is correct, it may be concluded that the existence of the ductus venosus is required by the termination of the umbilical and portal veins in opposite ends of the sinus intermedius. How, then, is the foetal pig able to dispense with the valvular function of the ductus venosus? It appears likely either that the umbilical and portal vein pressures are, in this species, at all times in equilibrium, or else that there exists another mechanism, as yet unknown, for maintaining this condition in spite of any tendency to an increase or decrease of pressure in one or other vein. The latter alternative seems the more probable one.

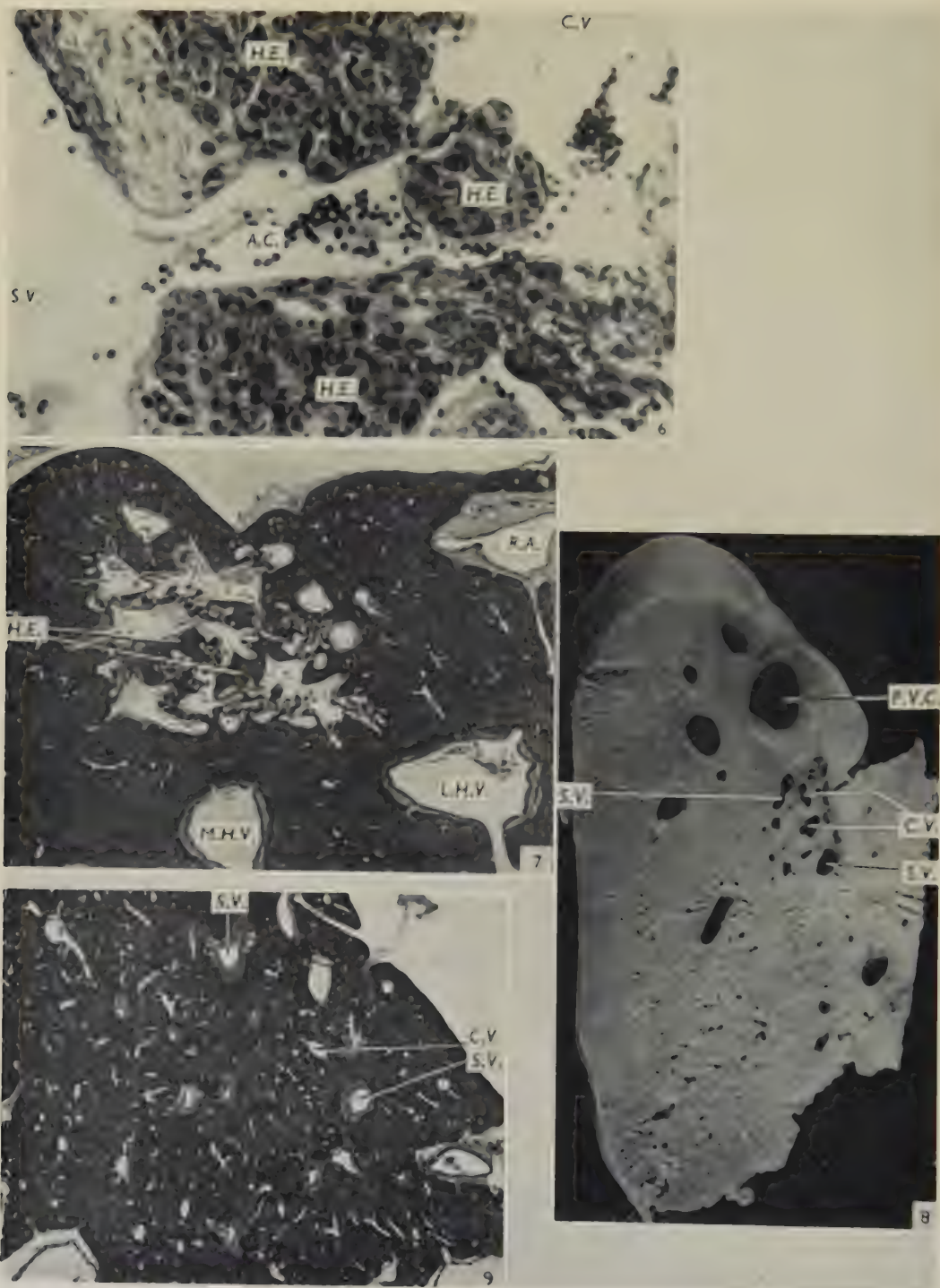
SUMMARY

The fate of the ductus venosus of the pig has been investigated in a series of ten specimens, ranging from an embryo of 14 mm. crown-rump length to a 14-day-old pig.

At the 14 mm. stage, the ductus venosus arises from the left end of the sinus intermedius and terminates in the left hepatic vein. Communications, which have been termed anastomotic channels, begin to appear between the ductus and the vessels supplying the neighbouring parts of the liver at about the 18 mm. stage. The caudal end of the ductus is obliterated between the 24 and 47 mm. stages, while the cranial portion of the ductus persists and continues to open into the left hepatic vein. The ductus, which is thus blind caudally, receives its blood through the plexus of anastomotic channels.

These anastomotic channels are at first relatively wide. They are always thin-walled, so that the blood in them comes into intimate contact with the surrounding hepatic epithelium. As development proceeds, the anastomotic channels become longer and narrower. It seems that eventually they become the sinusoids of the masses of hepatic tissue which separate the vessels supplying the plexus from the vessels draining it, all of which open into the persisting cranial portion of the ductus venosus. The end-result, which is probably attained before birth, is that the ductus venosus has been transformed into a hepatic vein, which drains its own territory of the liver and is a radicle of the left hepatic vein.





DICKSON—THE DUCTUS VENOSUS OF THE PIG

It is suggested that metabolic conditions in the pig require that all the blood returning from the placenta should come into relationship with the hepatic epithelial cells before release to the general circulation. It is further suggested that there probably exists in the circulation of the foetal pig an unknown mechanism for keeping the umbilical and portal vein pressures in equilibrium, for this is the function tentatively ascribed to the ductus venosus of those species in which the vessel persists until birth.

I am greatly indebted to Prof. J. D. Boyd and Prof. J. J. Pritchard for advice and guidance, and to Dr W. R. M. Morton, who drew the diagram from which the text-figure is reproduced, for constant encouragement. I am also indebted to Mr J. A. F. Fozzard and Mr J. F. Crane for assistance in preparing the illustrations.

REFERENCES

- BARCLAY, A. E., FRANKLIN, K. J. & PRICHARD, M. M. L. (1944). *The Foetal Circulation*. Oxford: Blackwell Scientific Publications.
- BARRON, D. H. (1942). The 'sphincter' of the ductus venosus. *Anat. Rec.* **82**, 398.
- CHACKO, A. W. & REYNOLDS, S. R. M. (1953). Embryonic development in the human of the sphincter of the ductus venosus. *Anat. Rec.* **115**, 151-174.
- DAWES, G. S., MOTT, J. C. & WIDDICOMBE, J. G. (1954). The foetal circulation in the lamb. *J. Physiol.* **126**, 563-587.
- HUZLY, A. (1942). Zur Anatomie (Bildung und Rückbildung) des Ductus venosus Arantii. *Anat. Anz.* **93**, 1-15.
- KLAGES, C. (1931). Anatomische Untersuchungen des Gefäßeverlaufs der Leber neugeborener Schafe und geburtsreifer Rinder. *Morph. Jb.* **68**, 301-324.
- PALTAUF, R. (1888). Ein Fall von Mangel des Ductus venos. Arantii. *Wien. klin. Wschr.* **1**, 165-167.
- REX, H. (1888). Beiträge zur Morphologie der Säugerleber. *Morph. Jb.* **14**, 517-617.
- REYNOLDS, S. R. M. (1951). Arterial and venous pressures in umbilical cord of the sheep and nature of venous return from the placenta. *Amer. J. Physiol.* **166**, 25-36.
- SCHNEIDER, H. (1937). Eine interessante Anomalie im Verhalten des Ductus Arantii. *Z. ges. Anat.* **1**, *Z. Anat. EntwGesch.* **107**, 319-352.

EXPLANATION OF PLATES

PLATE 1

- Fig. 1. A transverse section through the liver of an 18 mm. pig embryo. $\times 30$. The section shows the ductus venosus and the ramus omentalis arising from the sinus intermedius.
- Fig. 2. A transverse section through the liver of a 20 mm. pig embryo. $\times 30$. The section shows two anastomotic channels, separated by a septum of hepatic epithelium, connecting the ramus omentalis and the ductus venosus.
- Fig. 3. A transverse section through the liver of a 24 mm. pig embryo. $\times 25$. The section shows a large anastomotic channel connecting the ramus omentalis and the ductus venosus.
- Fig. 4. A transverse section through the liver of a 47 mm. pig foetus. $\times 40$. The section shows an anastomotic channel connecting a branch of the ramus angularis and the persisting cranial part of the ductus venosus.
- Fig. 5. A manually cut transverse section through the liver of a 100 mm. pig foetus. $\times 3$. The illustration shows the plexus of vessels (arrow) which is replacing the caudal part of the ductus venosus.

PLATE 2

- Fig. 6. A transverse section through the liver of a 210 mm. pig foetus. $\times 350$. The section shows an anastomotic channel connecting a supplying vessel to a collecting vessel, and illustrates the thinness of the wall of the anastomotic channel.
- Fig. 7. A transverse section through the liver of a 210 mm. pig foetus. $\times 10$. The section shows the masses of hepatic epithelium lying among the vessels of the plexus which is replacing the caudal part of the ductus venosus.

Fig. 8. A manually cut transverse section through the right side of the liver of a 1-day-old pig. $\times 2$. The illustration shows that the supplying vessels of the plexus are separated from the collecting vessels by a considerable amount of hepatic epithelium.

Fig. 9. A transverse section through the liver of a 14-day-old pig. $\times 10$. The section shows that the supplying and collecting vessels of the plexus are even further separated from one another by hepatic epithelium.

Key to lettering

A.C. anastomotic channel; *C.V.* collecting vessel; *D.V.* ductus venosus; *H.E.* hepatic epithelium; *L.H.V.* left hepatic vein; *M.H.V.* middle hepatic vein; *P.V.C.* posterior vena cava; *R.A.* ramus angularis; *R.O.* ramus omentalis; *S.V.* supplying vessel; *X*, branch of ramus angularis.

THE LEVATOR PALATI MUSCLE

By R. FRANCE ROHAN AND L. TURNER

Anatomy Department, University of Manchester

An attempt to solve difficulties encountered in the dissecting room by reference to the standard text-books revealed a lack of agreement concerning the origin of the levator palati muscle. Eustachius (1707) was the first to mention this muscle, placing the pharyngotympanic tube 'between the two muscles of the fauces or throat'; there is little doubt that he meant the tensor and levator palati. Soemmering (1796) placed the levator anterior to the carotid canal, at the junction between the bony and fibro-cartilaginous parts of the tube. Blakeway (1914) stated that the levator is not medial to the tube at its origin, and our findings have in the main agreed with his, but not with the current teaching.

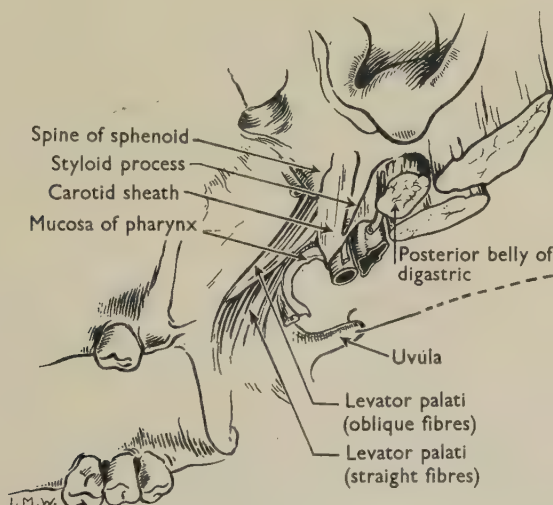


Fig. 1. The soft palate has been retracted and partly detached to display the levator palati.

To verify or disprove these varied opinions, we dissected four foetal and sixteen adult specimens, and have since confirmed our findings in ten further specimens.

We found that the levator palati arises:

(1) By a small tendon from a rough impression on the inferior surface of the petrous temporal bone, immediately in front of the inferior orifice of the carotid canal. The precise relationship of this area to the carotid canal varies in different specimens but is never medial to it.

(2) By a group of fleshy fibres from a sheet of fascia which depends from the vaginal plate and forms the upper part of the carotid sheath (Fig. 1). This origin has not been described before, but could be demonstrated in every case by careful dissection.

(3) By a very few fleshy fibres from the under surface of the fibro-cartilaginous part of the tube. The fibres which arise here are directly continuous with those which arise in front of the carotid canal. Moreover, the fleshy belly of the muscle lies below the tube, and only crosses over to its medial side at the level of the medial pterygoid plate.

Finally, the muscle consists of two parts, an infero-medial consisting of straight fibres, and a supero-lateral consisting of oblique fibres (Fig. 1). The two are separated by a small vessel and some fibrous tissue. In this connexion it is interesting to recall that, according to Baxter (1953), the levator palati develops in two parts from the third branchial arch—one from those cells of the palatal fold which reach the hamular process, and the other from the 'skew' fibres of the tubal portion of the fold.

We should like to thank Prof. G. A. G. Mitchell, in whose department this work was done, for his kindly advice and encouragement, and Miss I. M. Wright for the drawing. We are particularly indebted to Mr J. Kennaway, of Messrs Longmans Green and Co., Ltd., for his kindness in providing information from some early editions of *Gray's Anatomy*.

REFERENCES

- BAXTER, J. S. (1953). In Frazer's *Manual of Embryology*, 3rd ed. p. 267. London: Ballière, Tindall and Cox.
- BLAKEWAY, H. (1914). Investigation into the anatomy of the palate. *J. Anat. Physiol.* **48**, 412.
- EUSTACHIUS, B. (1707). *Opuscula Anatomica. De Auditus Organis*, p. 139.
- SOEMMERING, S. T. (1796). *De Corporis Humani Fabrica*, **3**, 131. Ad Moenum, Varrentrappii et Wennere.

REVIEWS

Neuro-vascular Hila of Limb Muscles. By JAMES COUPER BRASH. (100 pp., 31 coloured plates, 19 references; 30s.) Edinburgh and London: E. and S. Livingstone Ltd. 1955.

This excellently produced book will be welcomed by those who are responsible for the care of patients with neuro-vascular injuries of the limbs. It goes far to meet the need for information on the presence or absence of a definite neuro-vascular hilum and on the sites of entry of nerves of supply of the limb muscles. The work is based on a series of most careful dissections and measurements made in the departments of anatomy of the Universities of Edinburgh, Aberdeen, Leeds and Glasgow. The numbers of cases in which a definite neuro-vascular hilum was found is given for each of the muscles studied, and the site of entry of the nerve or nerves is presented in two forms. The first gives this point in terms of general proportions of either the segment of the limb or of the muscle itself. The second is concerned with a more accurate measurement of the site of entry, information which is of particular importance in the care of cases of injury to peripheral nerves. Average measurements both for the limb and site of entry are recorded, but unfortunately there is no indication of the precise number of specimens studied or of the range of variability which was found. This part of the work is given in two tables in which the present observations are correlated with those of previous authors, and it is surprising to see that there is no reference to the most recent and substantial contributions made by Sunderland and his colleagues. The bold and attractive semi-diagrammatic illustrations are based on actual dissections, but in some cases they are misleadingly over-simplified and there are some inaccuracies. In future editions it is to be hoped that these will be corrected and that it will be possible to give more data on actual measurements for the benefit of future workers.

RUTH E. M. BOWDEN

Bone. An Introduction to the Physiology of Skeletal Tissue. By FRANKLIN C. McLEAN and MARSHALL R. URIST. (Pp. xii+182; 26 illustrations+1 plate; 5 tables; $5\frac{1}{2} \times 8\frac{1}{2}$ in.; \$6.00 or 45s.) University of Chicago Press; Cambridge University Press. 1955.

The short title of this attractive, slim volume recalls P. D. F. Murray's book on *Bones* (Cambridge University Press, 1936). The difference of singular and plural, however, makes the one complementary to the other; for, to quote the Introduction: 'This book is concerned with bone as a tissue rather than with its mechanical function in providing the skeletal support of the body or with the shape, adaptation, or development of individual bones.'

As the authors state, and as its title implies, it 'is not intended as a full-scale monograph on the physiology of bone'. But the table of contents may well serve as an indication of the scope of such a future work, for there are chapters on bone as a tissue, its histogenesis, the chemistry and crystallography of bone, the roles of enzymes and hormones (including ACTH and cortisone), electron micrography, the use of radioactive isotopes and the problem of ectopic bone formation.

Yet in all these discussions of ultra-modern methods of investigation it is interesting and refreshing to find that the 'osteoblast' and the 'osteoclast' are still to the fore and are held to retain the importance in respect of the formation and resorption of bone, albeit as a reversible process, implied in their classical names.

The chapter bibliographies indicate the intensive current interest in bone-problems. Although historical treatment is at a minimum, there are a few classical references beginning with Paget (1860), Ollier (1867) and Kölliker (1873)—with some surprising omissions,

e.g. Leriche & Policard (1928)—but of some 260 titles, 66 date from the 1940's and 140 from 1950 to 1954. This, then, is a very useful miniature monograph and source-book for right up-to-date investigations on the perennial topic of bone.

It should be added that this is the first title in a new series, *The Scientist's Library: Biology and Medicine*, under the general editorship of Prof P. P. H. De Bruyn, Chairman of the Department of Anatomy, University of Chicago. According to the notice on the dust-jacket, this series is 'designed to acquaint the professional biologist with the fruits of advanced research in areas of specialization other than his own'. But it is to be hoped that the concluding sentence of this 'blurb' may not prove to be *literally* true—for it reads: 'It is believed that this new series will fulfil a long-felt need for sophisticated (*sic*) works which bridge the gaps between the many and increasingly specialized biological disciplines.'

J. C. BRASH

Growth at Adolescence. By J. M. TANNER. (Pp. xii+212; 11 plates+56 figures; 32s. 6d) Blackwell Scientific Publications. 1955.

In the opening paragraph of his preface Dr Tanner points out that there is 'not a single text describing human growth and development from a biological point of view or giving access to the extensive original literature'. This book makes a comprehensive survey of many of the changes that take place in human beings, and to some extent in other animals, as they grow from a pre-pubertal age to maturity.

Dr Tanner spends very little time in the discussion of the nature and mode of representation of growth processes, and his first chapter is mainly devoted to examples of the acceleration in growth that takes place during puberty. Data concerning changes in various organs are examined, in addition to the striking changes that occur in stature and total weight. The second chapter gives an account of the development of the reproductive system during puberty, describing and illustrating five stages in the development of the genital organs and five pubic hair stages. The following chapter discusses sex differences that arise in the adolescent development of skeletal and soft tissues. Chapter 4 examines the possible advantages of considering growth relative to developmental rather than chronological age. Four alternative systems are considered: skeletal age, dental age, morphological age (size, height) and secondary sex character age.

Chapter 5 is the longest and possibly the most interesting part of the book. Here Dr Tanner discusses the factors that may influence the puberty spurt. He is convinced that the time of this spurt is related to the somatotype of the individual. Studies in the effect of nutrition on growth rate show that there is some evidence 'that the time of occurrence of the adolescent spurt is a more sensitive indicator of nutritional deficiency than is the growth rate at earlier periods'. There is no evidence that exercise increases the growth rate, but there has undoubtedly been a secular trend in some countries during the last century for puberty to occur at an earlier age. There are short discussions on the relationship between socio-economic class, climate and race on the onset of adolescence.

Chapters 6 and 7 examine the physiological and endocrinological changes occurring during adolescence. Changes in blood-pressure, body temperature, haemoglobin, respiratory rate, basal metabolic rate, blood sugar, etc., in addition to those occurring in the endocrine organs, are all discussed. Chapter 8 adds data on muscle performance.

Chapter 9 gives an account of the growth of mental ability, and Dr Tanner states categorically that: 'During adolescence there is no doubt that growth in mental ability, that is, in those intellectual functions assessed by tests of intelligence and special aptitudes, continues.' The discussion of this problem and that of behavioural changes is very brief. The last chapter, 'The Adolescent Spurt in Animals', gives a short account of some growth processes in non-human mammals.

This rather lengthy summary has been given to show the extensive field of work that has been surveyed by the author of this book. He has examined an enormous amount of literature, brought together data from many sources and made a very readable and

interesting book. Yet one puts it down with a certain feeling of disappointment and with the impression that the author has given inadequate thought to some aspects of his work.

When the biologist speaks of 'growth' he usually refers to the changes in size and shape of an organism or system with respect to changes in time. There is no reason why the word should not be given a more extended meaning, but Dr Tanner should at least give some better indication of his use of the word. On page 2 two graphs are shown: one of the height attained by a boy at different ages, and the second showing the rate of change of height of the same boy at different ages. These two graphs would usually be described as growth and growth-rate curves respectively. In the first paragraph of the chapter referring to these curves, Dr Tanner merely says: 'Growth is a form of motion; the upper curve is one of distance travelled, the lower of velocity.' It is difficult to see, for example, how the weight attained at different ages can be usefully considered as a form of motion, or the rate of change of weight as a velocity. Although Dr Tanner uses other types of growth curves he gives no discussion of the relative merits of these different ways of representing growth data and makes no reference to specific growth and specific growth rate curves as such. It is true that these questions have been examined thoroughly and explained clearly by Medawar, but no reference is made to this important work in Dr Tanner's select bibliography 'of the more modern and the better papers'.

It is perhaps a result of a lack of recognition of some of the problems that are involved in any discussion on growth that leads to some of the less satisfactory comments of the author. For example, on page 37, referring to the fact that 'the legs of the male are longer than those of the female relative to body length', Dr Tanner remarks: 'It results simply from the boys' spurt occurring later than the girls'; and on page 159 'the maturation of the hypothalamus has been put back so that maturation of the association areas of the cortex and other parts can take place first'. Such comments seem to me to have very little scientific value. In other places Dr Tanner is mistaken. On page 92, discussing the fact that present-day adults are taller than those of fifty years ago may have a genetical basis, Dr Tanner comments that such a theory 'presupposes the existence of some degree of dominance in the genes governing stature'. There is no need at all to postulate a dominant gene; the situation would result if the heterozygotes were tall, an example of heterosis. (There is a verbal slip on page 96. Dr Mackay's work related to 'East' and not 'West' African children.)

Throughout the book Dr Tanner stresses the necessity for longitudinal rather than cross-sectional growth studies, rightly emphasizing the fact that data relating to the growth of individuals can only be obtained by examining the same individuals at different ages. Dr Tanner is well aware that growth data can only be evaluated by using adequate statistical methods, and he must be congratulated for his persistence in the tedious task of computation. He provides perhaps too many correlation coefficients, and might have warned his non-statistical readers that this coefficient only measures the degree of association between variables *presumed* to be associated. Is it really worthwhile to compute a discriminant function for classifying human beings into males or females by a combination of fat and bone measurements?

These are criticisms that occur to one reader and perhaps they represent a very personal point of view. It must be emphasized that this book contains very valuable data and many interesting comments.

D. A. SHOLL

The Lung: Clinical Physiology and Pulmonary Function Tests. By J. H. COMROE, R. E. FORSTER, A. B. DUBOIS, W. A. BRISCOE and E. CARLSEN. (Pp. viii + 219; 9½ × 6¼ in.; 57 illustrations; 40s.) Year Book Publishers Inc. 1955.

In recent years several new methods have been invented for studying quantitatively the process of respiration. In addition to measuring the chest expansion and vital capacity, which until recently were almost the only measurements that could be made, it is now possible to measure the efficiency of respiration and the work expended in it, and even to

apportion this work into that needed to expand the lungs and thorax and that used in transport of gas through the airways. The process by which inspired air mixes with that already contained in the functional residual capacity can be followed, estimates can be made of the evenness of alveolar ventilation and of the uniformity of perfusion of the alveoli with blood, and even of the size of the barrier to diffusion between alveoli and blood. The results of some of these measurements can be correlated with the existence of certain types of disease of the lungs, and others may be able to be so correlated in the future. Such measurements, whether or not of diagnostic or prognostic usefulness, have given and will continue to give a better insight into the physiology of respiration. The majority of the methods and their evaluation are so recent that they can only be found by reference to the original papers, thus this book is perhaps the first of what may well be a series of volumes by respiratory physiologists. These writers have shown particular care in the method of presentation of their material and have taken pity on the physician who shudders at equations. He is catered for by a series of excellent schematic drawings, almost cartoon-like in their clarity, and useful data, equations and calculations are, in the double-edged words of the Preface, presented in an Appendix for the enjoyment of those who have difficulty with words and pictures. The section on clinical applications is written around investigations of ten cases illustrating pulmonary disease and demonstrating the use of physiological tests. There is much to interest the anatomist, and many will find the illustrations alone of use and as examples of one way of illuminating a text undoubtedly successful.

R. J. HARRISON

Segmental Anatomy of the Lungs. A Study of the Patterns of the Segmental Bronchi and Related Pulmonary Vessels. By E. A. BOYDEN. (Pp. xviii + 276; 12 plates and 125 text-figures; 11 × 7½ in., \$15.00). McGraw-Hill Book Co. Inc. 1955.

The advances in thoracic surgery have now caused one of the objects of the surgeon in pulmonary resection to be the conservation of as much normal lung tissue as circumstances allow. Fifteen years ago the anatomist could give the surgeon little accurate guidance on the disposition of bronchopulmonary segments or the distribution of branches of the bronchi and blood vessels. Clinico-anatomists of the earlier years, Ewart, Kramer and Glass, Nelson, Churchill and Belsey, and Foster-Carter among others, were all aware that careful dissection, injection and the making of casts could all make a contribution in providing the required information. Later came the work of Appleton, and Brock in this country, and of Jackson and Huber in America, work resulting perhaps from anatomist turned bronchoscopist, or vice versa, or a happy combination of both. Now we review the book, destined almost to be a classic from the start, written by Emeritus Professor Boyden, who has not only built on the earlier work and organized teams to concentrate on particular aspects, but has himself supervised their efforts and exhaustively dissected the lung himself for ten years. It is not just the approach that is important, but the large numbers of lungs examined that form the quantitative basis of the report must so increase its value that future observations could probably only modify detail or alter nomenclature. The latter is still perhaps a vexed problem, and after a concise historical review of the matter Boyden adopts the Jackson-Huber terminology except in one respect—the raising of the two branches of the anterior medial basal bronchus to the status of segmental bronchi. This is not perhaps the place to discuss nomenclature, nor to comment on Boyden's criticisms of the report of the Thoracic Society, but at least the disadvantage of the existence of albeit three nomenclatures is obvious, even though they may be indicative of the active work in the field. Chapters 3 to 8 consider the prevailing patterns and variations of the bronchial distributions, the segmental arteries and the veins of the lobes of each lung. The account is clear, exhaustive, and is profusely illustrated, giving wherever it is known both the quantitative and practical significance of any particular variant. The chapter on the development

of the lung includes some as yet unpublished work in collaboration with L. J. Wells on Streeter's horizons XVII to XIX, which has a bearing on terminology and possibly on the genetic origin of aberrance in pattern. This is followed by a section on the preparation of the lungs for respiration, but which deals only briefly with the nature of the alveolar lining and hardly at all with the results of more recent investigations; it is this chapter only which could perhaps have been more expanded. The book is expensive, but is beautifully produced and a credit to the author and his publishers.

R. J. HARRISON

Bibliographie der Menschenaffen (Schimpanse, Orang, Gorilla). By Prof. Dr HERMANN Voss. (Pp. viii + 163; D.M. 12.80.) Jena: Fischer Verlag. 1955.

In his foreword to this bibliography Prof. Voss writes: 'Die vorliegende Bibliographie umfasst das Schrifttum bis zum Erscheinungsjahr. Es wurde die gesamte Weltliteratur berücksichtigt, darunter auch die sowjetische, soweit sie mir zugänglich war.' The reviewer unfortunately cannot comment on the completeness of the survey of the Russian literature on the anthropoid apes. But for that of the rest of the world he can state that the bibliography represents only the first of first approximations to completeness. A survey of the lists readily shows numerous gaps, including many standard works. Thus there is no reference to Connolly's book on the primate brain, nor to Bailey, v. Bonin and McCulloch on the chimpanzee iso-cortex, nor to the Raven memorial volume on the gorilla, nor to Walker on the primate thalamus, nor to Fulton and Kennard on the sign of Babinski, nor to J. P. Hill on the development of primates, nor to Huber's monograph on the facial musculature, etc. Wood Jones and Le Gros Clark are represented by one item each! There are a number of gaps in the Zuckerman list. There is no reference to the Ruch bibliography. In fact, anyone depending on Prof. Voss's bibliography would miss much most important literature.

Further there are many irritating mistakes. Bailey and McCulloch, for example, appear as Balley and Culloch; J. S. Huxley in the bibliography appears under T. H. Huxley in the index; A. H. Schultz never appears in the index at all!

For one prepared to check the index and to add, in an interleaved copy, the omissions, the volume would ultimately be most valuable—the skeleton of a really useful compilation is there. In its present state, however, the bibliography cannot be recommended as more than an incomplete one.

J. D. BOYD

Handbuch der mikroskopischen Anatomie des Menschen, IV. Band, *Nervensystem*, 2. Teil. Bearbeitet von G. SCHALTENBRAND und E. DORN. (Pp. vi, 195; 176 figures; D.M. 68.) Berlin: Springer Verlag. 1955.

This publication forms the second part of the fourth volume (on the Nervous System) of the well-known von Mollendorf's handbook. An interval of twenty-seven years has elapsed since the appearance of the first part in 1928. Like its predecessor this part is comprised of monographs by different authors. Prof. Schaltenbrand contributes the first on the choroid plexus and meninges, whilst Dr Dorn is responsible for the second on the saccus vasculosus.

Notable contributions have been made by Prof. Schaltenbrand to the understanding of the meninges and choroid plexuses, and he writes with authority on his subject. All aspects of the development, macroscopic and microscopic appearances of the meninges and plexuses are fully treated in his monograph. About half of the total of 140 pages is devoted to a detailed consideration of the choroid plexuses and the author has brought together in this section much valuable information which is widely scattered throughout the literature. The function of the choroid plexuses is considered, and the evidence in favour of the different theories of cerebrospinal fluid production is critically assessed. English readers would probably like to see, if only for its historical interest, some reference to the early

work of Bevan Lewis (1877) on the perivascular spaces. The articles by Penfield & McNaughton (1940) on the innervation of the dura mater, and by Patek (1940) on the perivascular spaces would also appear to have merited notice.

The second monograph by Dr E. Dorn on the saccus vasculosus comprises some 40 pages. In it she considers the occurrence of the saccus in different fishes. The development, microscopic structure, blood supply and innervation of the organ are described. In her final section Dr Dorn discusses the theories concerning the possible functions of this curious structure.

The book is excellently illustrated, but it is a pity that in many instances the magnifications of the photomicrographs are omitted.

It is when one comes to consider the bibliography that this book falls below the high standard of its text. The bibliography is comprehensive, but its value would, in the reviewers' opinion, have been considerably enhanced had more care been taken in its compilation. The reader is entitled to expect from a standard work an extreme degree of accuracy in the preparation and proof-reading of the list of references. In this work the abbreviations used for the titles of journals conform to no recognized system and even those chosen are not employed consistently. Numerous errors and omissions are to be found in the spelling of authors' names, in their initials, in the year of publication of the journal and in the citation of page numbers. A few examples must suffice; p. 127, Cushing's Cameron Prize Lectures on *The Third Circulation and its Channels* are given a joint authorship by Cushing, Weed and Wegefarth; p. 131, Hughson, W. appears as Hugson in Weed and Hugson—incidentally Hughson was not a part author of the article quoted; p. 131, absorption is written as absolution; p. 134, Woollard is misspelt Wollard.

The criticisms which have been made are not intended to detract from the merit of this work as a valuable contribution to the literature on the nervous system. The price of the book is regrettably such as to place it beyond the purse of many anatomists.

J. W. MILLEN

D. H. M. WOOLLAM

NEURAL PATHWAYS IN LACTATION

BY J. T. EAYRS AND R. M. BADDELEY

Department of Anatomy, University of Birmingham

It has long been recognized that suckling plays an important part in maintaining lactation. The principal underlying factor was at one time thought to be the withdrawal of milk, for a progressive involution of mammary tissues occurs when intra-alveolar pressures remain high (Petersen & Rigor, 1932; Selye & McKeown, 1934). More recent evidence, however (for reviews see Folley, 1947; Cross, 1955), suggests that suckling influences the course of lactation, not so much through a local effect (though such may be present) as by way of a system of neuro-endocrine reflexes involving the hypothalamus and pituitary gland.

The essential participation of the nervous system in this regulatory mechanism was first shown by Ingelbrecht (1935) who cut the spinal cord in the rat and found that, while lactation ceased when suckling was restricted to nipples innervated from segments caudal to the site of lesion, it continued when nipples cranial to this level were available. Little further has been done, however, to elucidate the anatomy and mode of function of the nervous pathways involved. Such evidence as is available suggests that the sensory receptors, though unidentified, are located in the nipples (Hooker & Williams, 1940) and that, since total sympathectomy does not inhibit lactation (Bacq, 1932), stimuli arising in these receptors are unlikely to be conveyed through autonomic channels. Central pathways to the hypothalamus, and those by which the hypothalamus regulates pituitary activity, remain obscure.

The present paper describes experiments, a preliminary account of which has appeared elsewhere (Eayrs & Baddeley, 1955), undertaken to study the course of this pathway through the neuraxis.

MATERIALS AND METHODS

Animals

One hundred and four virgin rats of the inbred 'Birmingham' strain were used. Each rat was mated, and on the day following the birth of its litter (except where otherwise stated) was partially thelectomized, i.e. all nipples except two pairs were excised, the young being reduced in number to two males and two females. The gain in weight of these four rats during the first 16 days of life (23 days in the case of group 1) was used as a measure of the lactational performance of the mother. In experiments where the gains in weight of successive litters were compared, the doe was always remated with the same male.

Operational procedures

The rats were divided into eight groups. Groups 1 and 2 were treated as described above and underwent no further operation. In the remaining groups the lactating

ability of the doe was measured after the following lesions, summarized in Table 1, had been made to the nervous system under avertin anaesthesia.

(a) *Group 3. Exposure of spinal cord*

The meninges were exposed either by removing the ligamentum flavum between the laminae of the 6th and 7th cervical vertebrae, or by removing a pair of vertebral laminae in the thoraco-lumbar region. The dura mater was then punctured with a sharp-pointed knife and the incision widened with forceps.

Table 1. *Summary of operational procedures*

Group	No. of rats	Treatments	
		To nipples	To nervous system
1	8	Partial thelectomy; four pups reared on two pairs of nipples	None
2	12		None
3	6		Spinal cord exposed
4	7		Section of dorsal roots
5	12		Section of dorsal funiculi
6a	5		Section of lateral funiculi after thelectomy
6b	4		Section of lateral funiculi before thelectomy
6c	10	Partial thelectomy; three pups reared on three nipples—two abdominal and one inguinal on same side	As for 6a but smaller lesions
7	6		Combined section of dorsal and ventral funiculi
8a	7		Ipsilateral hemisection of the cord after thelectomy
8b	6		Contralateral hemisection of the cord after thelectomy
8c	21		Ipsi- and contralateral hemisection of the cord before thelectomy

(b) *Group 4. Section of dorsal roots*

The deep muscles of the back were retracted and the vertebral laminae removed from L2 to T7. The spinal theca was then opened, and six consecutive pairs of sub-jacent dorsal roots cut close to the spinal cord. The bleeding which usually occurred both from the bone and from the superficial veins of the spinal cord was controlled by applying pledgets of cotton-wool soaked in thrombin.

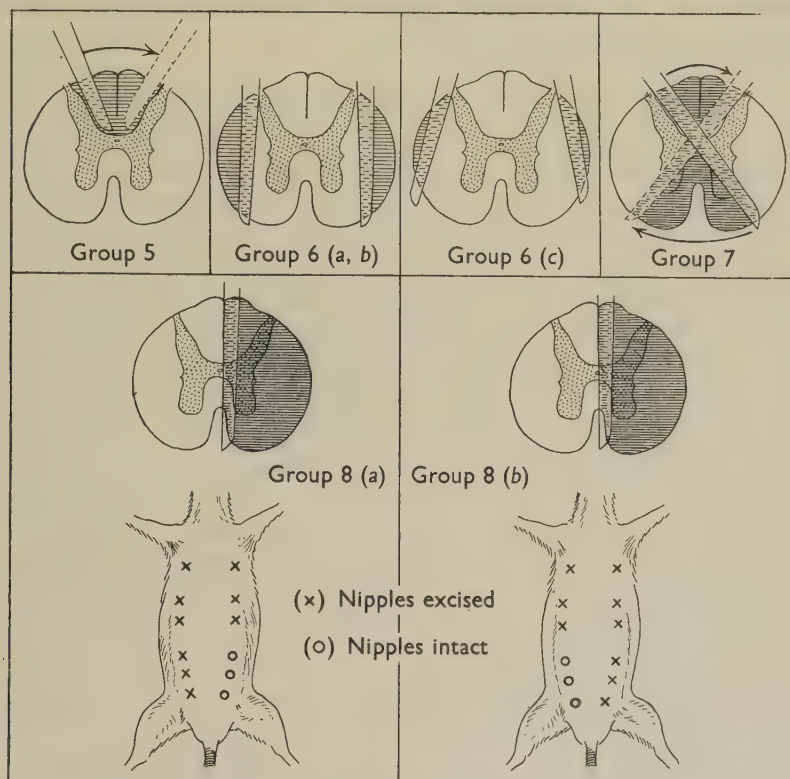
(c) *Groups 5–8. Section of tracts in spinal cord*

The spinal cord was exposed in the manner described for group 3, and bilateral lesions made with a discission knife (see Text-fig. 1) in the dorsal (group 5) and lateral (group 6) funiculi and in the dorsal and ventral funiculi combined (group 7). In the rats of group 8, the spinal cord was hemisected, the nipples of some of these animals being removed ipsilaterally, and in others contralaterally. Further details of these procedures, including the levels at which the several lesions were made, are, for convenience, given with the results.

Preparation of tissues

At the end of the experiments, the rats of groups 3–8 were killed in chloroform vapour. The central nervous system was removed and prepared for histological examination by Marchi's original method or by the Swank & Davenport (1935)

modification, representative celloidin sections being cut at 20μ through the spinal cord, medulla, pons and mid-brain. In addition, in the rats of group 8, that part of the vertebral column containing the lesion to the spinal cord was decalcified and embedded in paraffin wax. Longitudinal sections 15μ thick were cut and stained with cresyl violet or impregnated with silver (method of Romanes, 1950).



Text-fig. 1. Schematic diagrams showing manner in which lesions were made to spinal cord of rats in groups 5-8.

RESULTS

(a) Control operations—standard of lactational performance

(i) Thelectomy only

The results (Table 2a, b) serve to establish the pattern of growth of successive litters reared by the same female. They show that, in the absence of injury to the nervous system, such litters did not grow at the same rate, the mean weight of the second litter being significantly greater (11 % in group 1 and 13 % in group 2) than that of the first. The growth of the third litter did not, however, differ from that of the second. The rate of growth of these litters proved independent of which pairs of nipples were available for suckling.

(ii) *Mock operation*

Mock operation in the region of the spinal cord caused some impairment of lactation (Table 2c). The spinal theca in the rats of group 3 was opened on the day following the birth of the second litter, and although this litter grew slightly better than the first, the marked improvement in lactation characteristic of the unoperated rat (groups 1 and 2) was considerably reduced. The third litter grew slightly, but not significantly, better than the second.

(b) *Effect of bilateral lesions to the nervous system*(i) *Section of dorsal roots*

The dorsal roots were cut in seven rats (group 4) before mating and before any nipples had been removed. Two of these rats proved sterile; the other five were allowed

Table 2. *Lactational ability of rats—effect of partial thelectomy both alone and combined with lesions to the nervous system*

Serial	Group	No. of rats	Treatment	Mean increase in weight of litter of four rats from birth to 17 days old (g. rat)			Differences \pm standard error	
				Litter 1	Litter 2	Litter 3	Litter 1 ~ litter 2	Litter 2 ~ litter 3
(a)	1	8	Partial thelectomy	35.2	39.2	—	4.0 ± 1.66 $t = 2.425$ $P = 0.05-0.02$	—
(b)	2	12	Partial thelectomy	23.2	26.3	26.3	3.1 ± 0.46 $t = 6.682$ $P < 0.001$	Nil
(c)	3	6	Partial thelectomy	21.1	—	—	1.3 ± 1.21	1.0 ± 1.21
			Partial thelectomy combined with thecal incision	—	22.4	23.4	$t = 1.070$ $P = 0.4-0.3$	$t = 0.829$ $P = 0.5-0$
(d)	5	12	Partial thelectomy	22.6	—	—	2.6 ± 0.89	1.4 ± 0.89
			Partial thelectomy combined with section of dorsal funiculi	—	20.0	21.4	$t = 2.880$ $P = 0.02-0.01$	$t = 1.523$ $P = 0.2-0$
(e)	6c	8	Partial thelectomy	21.1	—	—	2.4 ± 0.80	—
			Partial thelectomy combined with section of lateral funiculi	—	18.8	—	$t = 2.999$ $P = 0.02-0.01$	—

to rear their full litters for a few days until it was established, by examining the stomachs of the young, that ample quantities of milk were being obtained. The extent of the anaesthetic region of the abdominal wall was then determined by pinching the nipples and surrounding skin with forceps under very light ether or avertin anaesthesia, and noting the presence or absence of a scratch reflex. All nipples outside this zone were then removed and the litter reduced to a size of one pup to each remaining nipple.

Lactation ceased altogether once the nipples outside the anaesthetic area had been removed, in spite of the fact that the remaining nipples were suckled vigorously and that the doe continued to nurse the young. After remating, two of these rats did succeed in rearing subsequent litters—one its third, and the other its fourth and fifth. This recovery coincided with a restoration of cutaneous sensitivity in the region of the inguinal nipples.

(ii) *Section of dorsal funiculi*

Lactation did not cease after section of the dorsal funiculi, but was impaired more severely than after mock operation alone (Table 2*d*). The operation was carried out at the level of C7 on the twelve rats of group 5 on the day following the birth of the second litter, which grew significantly less well (11 %) than did the first. The growth of the third litter showed a partial recovery to a level half-way between that of the first and the second.

(iii) *Section of lateral funiculi*

Small lesions made to the lateral funiculi were associated with an impairment to lactation comparable with that following section of the dorsal funiculi (Table 2*e*). Lactation ceased altogether, however, as a result of more extensive lesions (see Text-fig. 1) which at the same time interfered with micturition.

These observations were made on the nineteen rats of group 6 (see Table 1). Five of these (sub-group 6*a*) were rats whose performance had already been studied in group 1. Each had accordingly only four nipples at the time of operation. The lateral funiculi were cut at the level of T6 on the day following the birth of their third litter, and the litter was at the same time reduced to four in number. Three of these rats failed to rear their young altogether; the remaining two reared their litters, but

Table 3

	Operational procedure	Physical disabilities	Lactational performance
Sub-group 6 <i>b</i> (four rats)	Bilateral lesions, similar to those of non-lactating rats of sub-group 6 <i>a</i> , made <i>before</i> thelectomy	Paresis. Bladder dysfunction	Raised litters before partial thelectomy; lactation ceased after removal of all but the abdominal and inguinal pairs of nipples
Sub-group 6 <i>c</i> (ten rats)	Smaller bilateral lesions similar to those of lactating rats of sub-group 6 <i>a</i> , made <i>after</i> thelectomy	Paresis. No bladder dysfunction	Eight rats raised litters but less successfully than pre-operationally (Table 2 <i>e</i>). Two rats failed to lactate

the growth of their young was considerably less than that of their previous litters. All suffered from physical disabilities, e.g. paresis of the hind-limbs, disturbed gait and retention of urine, but the condition of the two which lactated was better than that of those which did not.

As a result of these findings, two further experiments carried out to determine whether the failure of lactation was directly due to the interruption of spinal pathways or was caused indirectly by the inability of the doe to nurse the young. These gave the results shown in Table 3.

It may be inferred from the combined results of these experiments: (i) that the arrest of lactation in animals with extensive lesions in the lateral funiculi of the spinal cord is directly due to interference with the afferent nervous pathways concerned with maintaining lactation, rather than to indirect causes; and (ii) that these pathways are situated deep in the spinal cord and are anatomically closely associated with those concerned in the control of the bladder.

(iv) *Combined section of dorsal and ventral funiculi*

This procedure varied in its effect, resulting either in an impairment of lactation considerably more severe than that which followed section of the dorsal funiculi alone, or in complete arrest of lactation. Lesions were made to the spinal cords of the six rats used (group 7) at the level of C7. Three of these rats failed to raise their second (post-operative) litter. The other three raised their second litter, but in all cases the young gained less weight (34 %) than those of the first (pre-operative) litter. All rats but one, however, reared their third litters but the growth of these litters was markedly depressed (40 %) relative to that of the first.

(c) *Effect of unilateral lesions—total hemisection of the spinal cord*

The effect of complete hemisection depended upon whether nipples were available on the same side as that of the lesion (ipsilateral hemisection) or on the opposite side (contralateral hemisection—see Text-fig. 1). With a few exceptions rats which had undergone ipsilateral hemisection failed to lactate while those with contralateral lesions succeeded in raising their litters.

Thirty-four rats in group 8 were used for this experiment. Of these, 13 (sub-groups 8a and 8b) had successfully reared a reduced litter of three young on three nipples (two abdominal and one inguinal on the same side) before operation. In seven rats (sub-group 8a), the spinal cord was cut ipsilaterally on the day following the birth of the second litter which was at the same time reduced to three in number. Six of these rats failed to rear their litters; one succeeded, but its young at 17 days old weighed considerably less than did those of its first litter. Contralateral lesions were made to the spinal cords of the six rats of sub-group 8b. Five of these rats raised their litters (though less successfully than their first litters), and one failed.

In the remaining twenty-one rats (sub-group 8c), which had previously reared a full-sized litter satisfactorily, the experimental procedure was varied, the spinal cords being hemisected (10 at cervical, 11 at thoracic level) and the animals returned to their litters without interference with the nipples. When lactation was firmly re-established, and when the rat had recovered from any obvious motor disability and was nursing the young in a well-made nest, partial thelectomy was performed leaving only the abdominal and inguinal nipples on one side. The size of the litter was at the same time reduced to three. Irrespective of whether the lesion was made in the cervical or thoracic region of the cord, all the rats with contralateral lesions (7) continued to lactate satisfactorily. On the other hand, the young of ten out of the fourteen rats which had received ipsilateral lesions died of inanition, no milk being seen in their stomachs later than 12 hr. after thelectomy.

These findings are thus substantially similar to those for sub-groups 8a and 8b, with which they may therefore be combined as under:

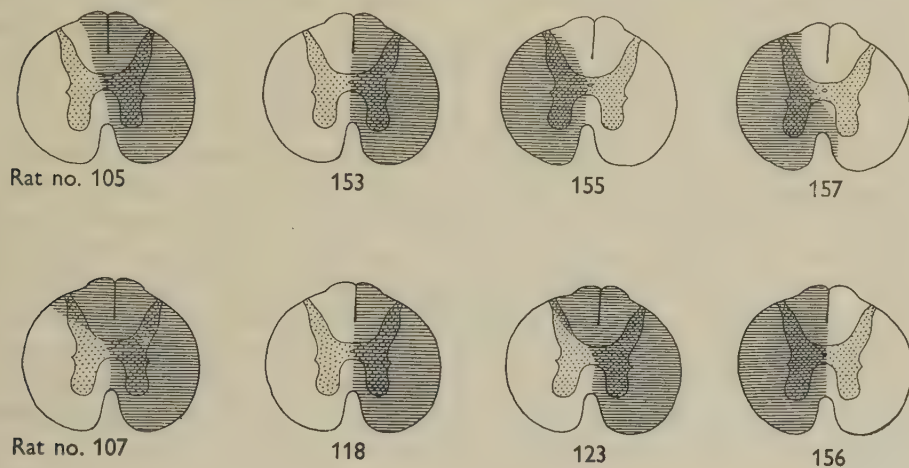
Lesion	Lactated	Failed to lactate	Total	Statistics
Ipsilateral hemisection	5	16	21	$\chi^2 = 12.45$
Contralateral hemisection	12	1	13	$P < 0.01$

It may be inferred from these figures that the stimuli concerned in maintaining lactation are for the most part conveyed ipsilaterally through the spinal cord. At the

same time it was not possible, as a result of histological examination of the site of lesion (see Text-fig. 2), to explain the lactational ability of the five rats which reared their young after ipsilateral hemisection of the spinal cord in terms of inadequate destruction of nervous tissue. It seems likely, therefore, that the pathway is bilaterally represented, containing a contralateral component which is adequate to maintain lactation after interruption of the main pathway, but whose destruction has only a depressing effect.

(d) *Histological examination*

A study of the degeneration which had occurred in the spinal cord and brain stem, other than confirming the extent of the various lesions, provided little new information. The tissues of the mock-operated rats (group 3) showed no sign of



Text-fig. 2. Comparison of extent of ipsilateral lesions to the spinal cord (hatched) in: above—four rats which failed to lactate; below—four rats (typical sample) which lactated satisfactorily. The damage was assessed from histological preparations taken through the site of lesion. Since the dorsal funiculi are not essential for maintaining lactation, there are no obvious differences which could explain the difference in lactational ability in terms of an undamaged ipsilateral pathway (see text).

degeneration anywhere in the neuraxis. Those of the remainder (Pl. 1) showed the expected ascending and descending degeneration in the spinal cord. The former could be traced to the gracile and cuneate nuclei in tissues where the lesions had involved the dorsal funiculi (Pl. 1, figs. 1, 3), and through the dorsal and ventral spinocerebellar tracts to the white matter of the cerebellum when the lateral and ventral funiculi of the cord had been interrupted (Pl. 1, figs. 2, 3). Diffuse granulation could often be seen in the reticular formation of the lower brain stem which was more concentrated in the region occupied by the medial lemniscus, but although spinothalamic involvement might have been expected in lesions to both the lateral and ventral funiculi, no consistent degeneration was ever seen above the level of the pons. The absence of such degeneration was not due to the fact that, in the rats of groups 5-7, a considerable time (2-3 months) had lapsed between operation and autopsy,

for in rats which had undergone hemisection of the spinal cord, and whose tissues had been prepared at the optimum time of 14 days after operation, the findings were similar (see Pl. 1, fig. 3).

DISCUSSION

The experimental findings help to elucidate the neural pathways by which lactation is maintained by suckling. It is clear, as would be expected, that the pathway enters the spinal cord at segmental level by way of the dorsal roots. Its subsequent course within the spinal cord however, although strongly indicated, cannot be defined precisely.

The results for the rats of group 8 (hemisection of the spinal cord combined with unilateral removal of nipples) show that, although a weak contralateral component may be present, the pathway follows a predominantly ipsilateral course; but at the same time it does not seem that the function of maintaining lactation can be assigned to either of those systems which are classically regarded as 'uncrossed'. In the first place it would appear most improbable, both on anatomical and on functional grounds, that the spino-cerebellar tracts are implicated; and secondly, the findings argue against the possibility that the fasciculi gracilis and cuneatus are involved. For instance (i) the interruption of these tracts does not cause a complete arrest of lactation; (ii) the impairment which ensues is to some extent transient, and although more severe, is similar in type to that which follows exposure of the spinal cord without apparent damage to nervous tissues; and (iii) similar effects result from mild lesions to the lateral funiculi, the dorsal funiculi remaining intact. Hence, although the participation of the fasciculi gracilis and cuneatus cannot entirely be excluded, it is more likely that the effects of lesions to these tracts are the indirect results of non-specific trauma (e.g. that caused by interference with the vascular supply of neighbouring structures) rather than to the interruption of specific pathways.

It seems therefore that the pathway must be related to the spino-thalamic/spino-tectal system within which the body is believed to be represented bilaterally (Walker, 1940). The present results do not, however, conform with the generally accepted organization of the spino-thalamic projections in so far as, in man, only a small minority of fibres is thought to be uncrossed. Hence unilateral damage to the system would be expected to produce its most severe effects contralaterally while the reverse is the case where the fibres responsible for maintaining lactation are concerned. It is unlikely that this anomaly can be explained by the ipsilateral ascent, for a few segments, of either first or second order fibres, for unilateral lesions in the cervical region were similar in their effect to those made at thoracic level. It would therefore appear either that all the fibres which regulate lactation are contained in the small uncrossed component, or that the spino-thalamic projections as a whole are differently organized in the rat from those in man. Evidence in support of the latter view is provided by the observation that, after hemisection of the cord in the rat, a squeak can be elicited by pricking either foot. It is thus clear that this operation by no means interrupts the pathway mediating the perception of painful stimuli, as might be expected from the usual effects of unilateral spino-thalamic tractotomy in man.

The general position within the spinal cord occupied by the fibres concerned in lactation may be deduced from a comparison of the site and extent of lesions which arrest lactation and those which do not. As argued above, the dorsal part of the cord can probably be excluded. Lesions to both the lateral and ventral funiculi vary in their effect. When, as shown by histological preparations, the lesion to the lateral columns is small, or occurs predominantly in the dorsal part of these columns, lactation is affected no worse than it is by lesions to the dorsal funiculi. Much the same considerations apply to injury to the medial part of the ventral funiculi. When, however, lesions to the lateral funiculi are made more deeply, or when those to the ventral funiculi are extended laterally, lactation ceases. From such observations it may be inferred that the pathway is predominantly localized deep in the lateral portion of the cord. The fibres appear to be intermingled with those concerned with the control of the pelvic viscera, and it is of interest that the lesions which prevent lactation in the rat are largely co-extensive with those which interfere with micturition in the cat (Barrington, 1933) and with defaecation in man (Nathan & Smith, 1953). It might be expected, by analogy with the topical arrangement of fibres in the human spino-thalamic tract proposed by Walker (1940), that fibres mediating sensation from the abdominal wall would be localized rather more superficially at thoracic and cervical levels than is suggested by the results of the present experiments. In the rat, however, the cortico-spinal tracts are carried in the dorsal funiculi, and the consequent difference in the organization of the lateral columns between the rat and man may account for the apparent discrepancy.

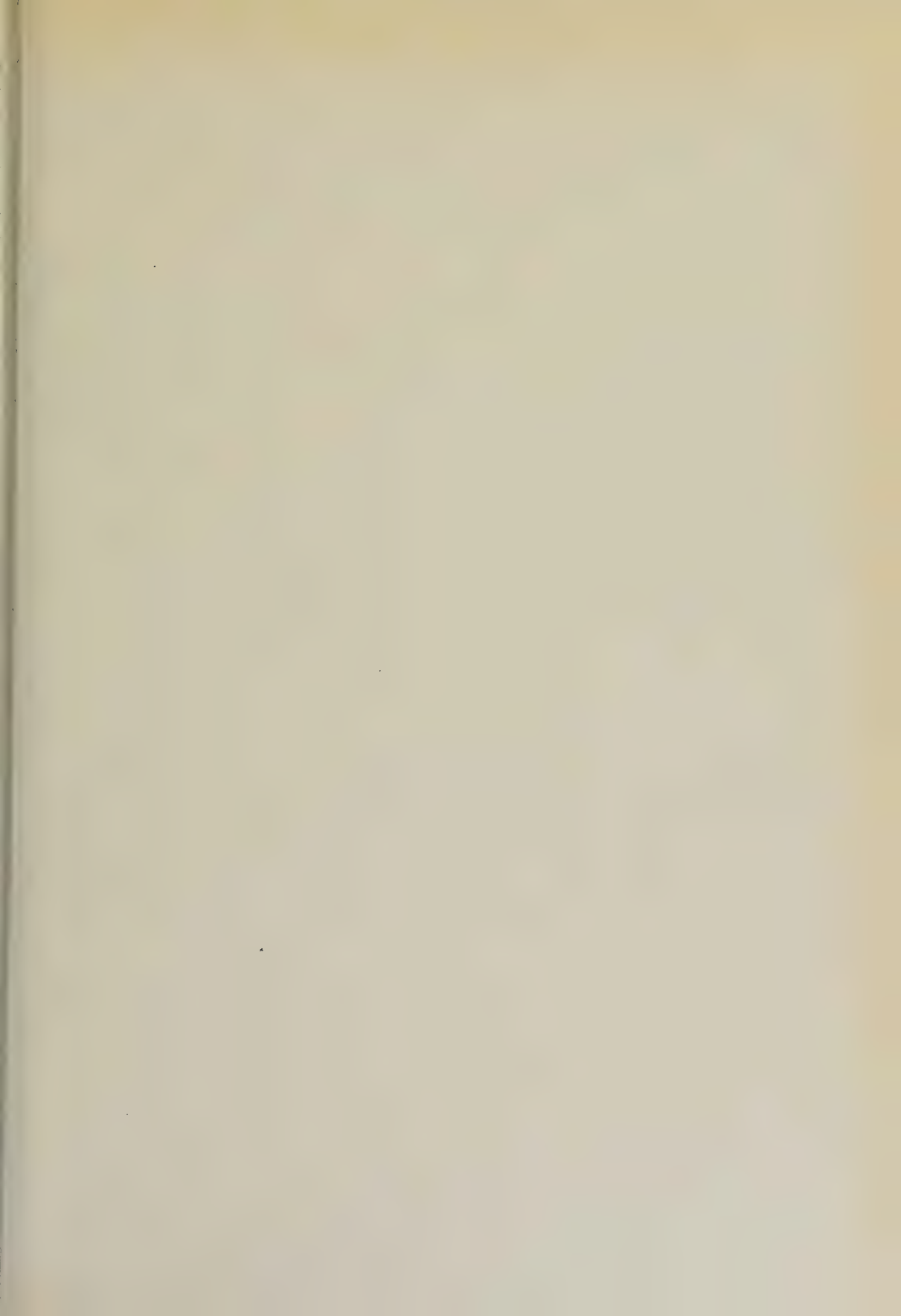
Little information concerning the localization in the brain stem of the pathway subserving lactation could be obtained from the study of histological material. In fact, from the absence of any consistent degeneration in Marchi preparations of the mesencephalon, even in the tissues of animals killed at the optimum time of 14 days after operation, it is clear that the Marchi technique is unsuitable for studying the course of this pathway in the rat. It would appear either that (i) the fibres are unmyelinated, (ii) they are not fasciculated in any discretely organized tract but are widely dispersed throughout the reticular formation, or (iii) that the pathway consists of multiple relays, in which connexion attention has already been drawn to the small number of spino-thalamic fibres which reach the thalamus direct in primates (Walker, 1940). An examination of the effects of small electrolytic lesions discretely placed throughout the neuraxis will probably yield more satisfactory information concerning the course of this pathway than will degeneration studies. Work on these lines which, it is hoped, will help to elucidate both the mode of termination and function of the ascending pathways now studied at lower levels, is already in progress. Incidental observations already made during the present experiments suggest that these pathways influence the activity both of the adeno- and of the neuro-hypophysis. For example, the oxytocic principle of the posterior pituitary, a hormone capable of maintaining lactation after lesions to the supra-optico hypophysial tract (Cross & Harris, 1952), section of the pituitary stalk (Harris & Jacobsohn, 1952) or when given together with anterior lobe extracts after hypophysectomy (Gomez, 1940), will restore lactation in rats after it has ceased as a result of lesions made to the spinal cord. Injections of this hormone have been effective, however, only for a period of up to 48 hr., after which lactation ceased

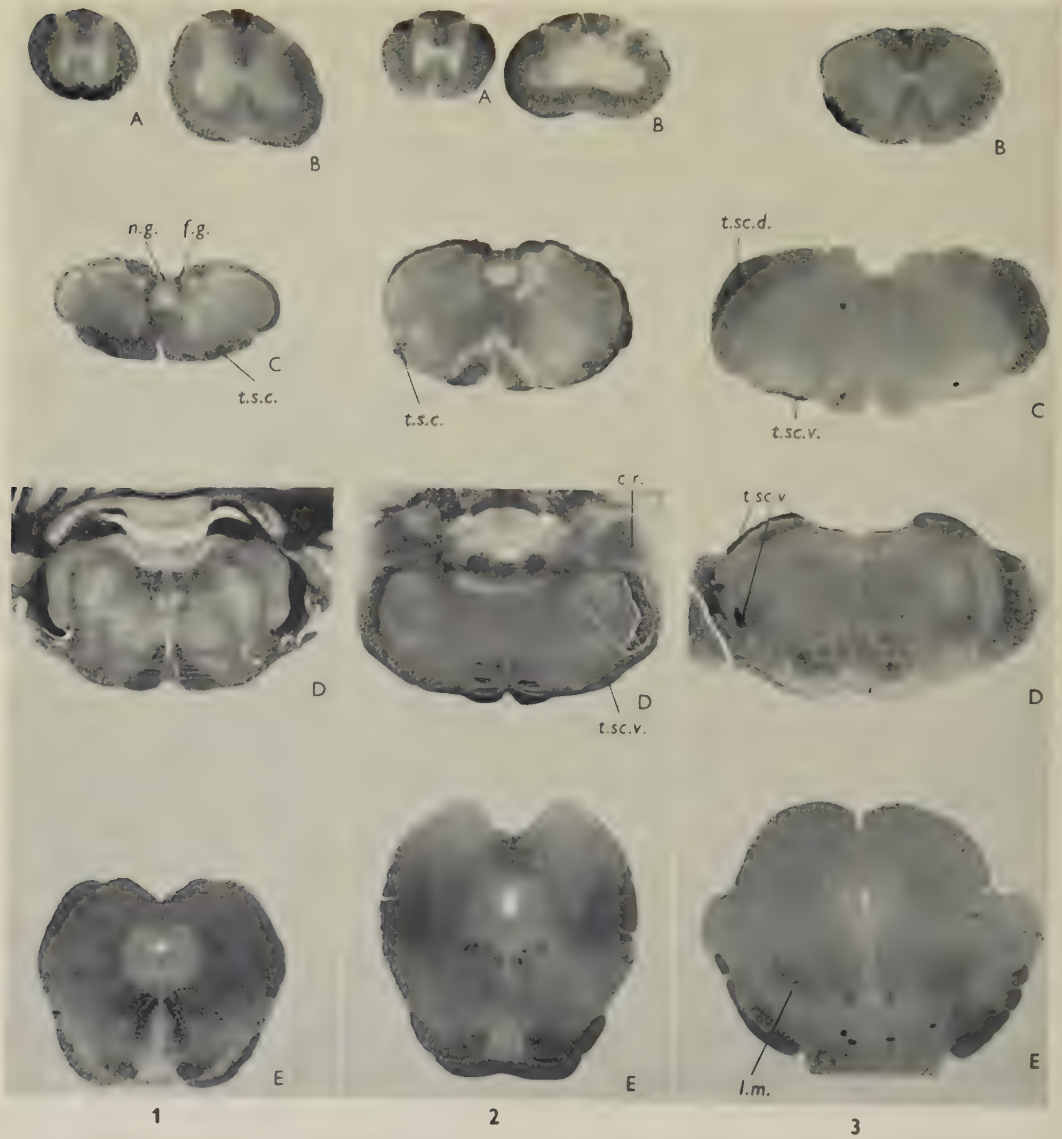
altogether. It may tentatively be postulated from such findings that both lobes of the pituitary cease to exert their characteristic influence over lactation (see Folley, 1947) when the arrival of stimuli originating in the nipples is interrupted. The secretions of the neuro-hypophysis, which are responsible for the ejection of milk, can be replaced by exogenous oxytocin, but no milk is secreted once the galactopoietic principles of the adeno-hypophysis have been exhausted.

A further observation, which may prove to be of considerable importance for understanding the neural mechanisms in lactation, is that the return of the ability to raise young coincided with the re-establishment of sensitivity in the region of the inguinal nipples. Since the sensory innervation of this region was interrupted central to the dorsal root ganglion, it follows that the return of sensation must have been due to the ingrowth of nerve fibres from segments below the level of the lesion which would not normally be concerned with lactation. If more fully substantiated this finding would suggest that the stimulus of suckling may maintain lactation not so much through an anatomically discrete pathway as by setting up a specific rhythm of discharge determined by an interaction between (i) the form of stimulus, (ii) the properties of the receptors, and (iii) excitable relationships at central synapses. Such a possibility has already been suggested by studies on the mechanism underlying cutaneous sensibility to temperature change (Lele, Weddell & Williams, 1954), a modality which is also mediated by the spino-thalamic system.

SUMMARY

1. A study has been made of the nervous pathways through which lactation is maintained by the act of suckling.
2. Lactation, as measured by the growth of the litter, is slightly impaired as a result of exposing the spinal cord, and rather more severely when the dorsal columns are cut. In both these instances, however, there is a recovery in lactational performance though not to the preoperative level.
3. Lactation is also impaired by mild bilateral lesions to the lateral funiculi of the cord and is inhibited by more severe lesions in this region. It is also prevented by cutting the dorsal roots of the nerves supplying the segments in which the suckled nipples are situated.
4. With few exceptions lactation ceases following hemisection of the spinal cord when the only nipples available for suckling are on the same side as the lesion. Hemisection does not cause arrest of lactation when nipples are suckled on the side opposite to that on which the lesion is made.
5. It has not been possible to trace the course of the pathways interrupted by lesions to the spinal cord from a study of Marchi preparations.
6. It is inferred from these findings that the pathway by which the suckling stimulus maintains lactation enters the central nervous system by the dorsal roots and ascends in the spinal cord deep in the lateral funiculus of the same side. This pathway to the diencephalon is either indirect, unmyelinated, or not fasciculated but widely dispersed throughout the reticular formation.





REFERENCES

- BACQ, Z. M. (1932). The effect of sympathectomy on sexual functions, lactation and the maternal behaviour of the albino rat. *Amer. J. Physiol.* **99**, 444-453.
- BARRINGTON, F. J. F. (1933). The localization of the paths subserving micturition in the spinal cord of the cat. *Brain*, **56**, 126-148.
- CROSS, B. A. (1955). The posterior pituitary gland in relation to reproduction and lactation. *Brit. Med. Bull.* **11**, 151-155.
- CROSS, B. A. & HARRIS, G. W. (1952). The role of the neurohypophysis in the milk-ejection reflex. *J. Endocrin.* **8**, 148-161.
- EAYRS, J. T. & BADDELEY, R. M. (1955). Le système nerveux et la lactation. *Resumés des Communications. VI^e Congrès Fédératif International d'Anatomie, Paris.*
- FOLLEY, S. J. (1947). The nervous system and lactation. *Brit. Med. Bull.* **5**, 142-148.
- GOMEZ, E. T. (1940). Effect of post-hypophyseal extract on lactation in hypophysectomized post-gravid rats. *J. Dairy Res.* **23**, 537-538.
- HARRIS, G. W. & JACOBSON, D. (1952). Functional grafts of the anterior pituitary gland. *Proc. Roy. Soc. B*, **139**, 263-276.
- HOOKE, C. W. & WILLIAMS, W. L. (1940). Retardation of mammary involution in the mouse by inhibition of the nipples. *Yale. J. Biol.* **12**, 559-564.
- INGELBRECHT, P. (1935). Influence du système nerveux central sur la mamelle lactante chez le rat blanc. *C.R. Soc. Biol., Paris*, **120**, 1369-1371.
- LELE, P. P., WEDDELL, G. & WILLIAMS, C. M. (1954). The relationship between heat transfer, skin temperature and cutaneous sensibility. *J. Physiol.* **126**, 245-256.
- NATHAN, P. W. & SMITH, M. C. (1953). Spinal pathways subserving defaecation and sensation from the lower bowel. *J. Neurol. Psychiat.* **16**, 245-256.
- PETERSEN, W. E. & RIGOR, T. V. (1932). Relation of pressure to rate and quality of milk secreted. *Proc. Soc. Exp. Biol., N.Y.*, **30**, 254-256.
- ROMANES, G. H. (1950). The staining of nerve fibres in paraffin sections with silver. *J. Anat., Lond.*, **84**, 104-115.
- SELYE, H. & McKEOWN, T. (1934). Further studies on the influence of suckling. *Anat. Rec.* **60**, 323-332.
- SWANK, R. L. & DAVENPORT, H. A. (1935). Chlorate-osmic-formalin method for staining degenerating myelin. *Stain Tech.* **10**, 87-90.
- WALKER, A. E. (1940). The spinothalamic tract in man. *Arch. Neurol. Psychiat., Chicago*, **43**, 284-298.

EXPLANATION OF PLATE

Extent of degeneration in spinal cord and brain stem following various lesions to the spinal cord. A: below level of lesion; B-E: in ascending order above level of lesion.

Fig. 1. Degeneration following section of dorsal and ventral funiculi. The preparation was made 3 months after the lesions, and much of the original granulation has presumably cleared up. Ascending degeneration in the medulla is confined to the fasciculi and nuclei gracilis (*f.g.* and *n.g.*) and to the spino-cerebellar tracts (*t.s.c.*).

Fig. 2. Degeneration following combined section of dorsal and ventral funiculi (lesion 3 months old). The only marked degeneration is in the spino-cerebellar tracts (*t.s.c.*) in the medulla and in the ventral spino-cerebellar tracts (*t.sc.v.*), restiform body (*c.r.*) and white matter of the cerebellum at the pontine level. None can be seen in the mid-brain.

Fig. 3. Degeneration following hemisection of the spinal cord. Preparation made 14 days after lesion, but even so degeneration is confined to the dorsal funiculi and spino-cerebellar tracts (*t.sc.d.* and *t.sc.v.*). The slight unilateral granulation in the medial lemniscus (*l.m.*) at the level of the mid-brain was not found consistently.

SOME CHARACTERISTICS OF MYELINATED FIBRE POPULATIONS

By T. A. QUILLIAM

Department of Anatomy, University College London

INTRODUCTION

That peripheral nerves consist of aggregations of separate microscopic fibres was first realized by Monro (1779) and Fontana (1781), and many attempts have since been made to determine criteria by which these fibre populations could be satisfactorily characterized. Monro (1783) made a rough estimate of the diameter of the largest fibre he could visualize (*c.* 9/1,000ths of an inch), and a century later Schwalbe (1882) classified the myelinated fibres in the spinal roots of frogs into diameter size groups measured in microns. Using diameter as a criterion Gaskell (1886) was able to ascribe certain autonomic functions to specific myelinated fibres in dogs by tracing them to their source. Sherrington (1894), studying muscle nerves in monkeys and cats, showed by degeneration experiments that they contain a considerable proportion of fibres from the dorsal spinal roots, some of which are nearly as large in diameter as the greatest which come from the ventral spinal roots. Schiller (1889) was the first to use diameter measurements of whole myelinated fibre populations to estimate normal growth rates. He was followed by many other workers who have employed this method to gauge both normal growth and regrowth after trauma. Similar measurements have also been made in the study of the relationship of fibre diameter to function by means of electrophysiological methods which involve knowledge of the correlation between fibre diameter and conduction velocity (Erlanger & Gasser, 1937; Hursh, 1939; Kuffler, Laporte & Ransmeier, 1947; Kuffler, Hunt & Quilliam, 1951; Tasaki, Maruhashi & Mizuguchi, 1952).

In the present paper the absence of myelinated fibre tapering and branching over considerable distances is demonstrated in a cutaneous nerve, and the degree of individual variation with respect to total numbers of myelinated fibres and diameter size frequency distributions is determined in both a muscle nerve and a cutaneous nerve of the rabbit. In addition, the corresponding nerves of a sheep and a rat are examined, and the influence of the body weight on the myelinated fibre populations found within them is thus determined. The limits of accuracy of the methods used are explored and some additional ways of supplying relevant data are suggested.

The sural nerve and the nerve to the medial head of the gastrocnemius muscle (N.G.M.) were chosen for this work. Both are of easy access, but the former is purely cutaneous in distribution whilst the latter innervates only somatic muscle. The sural nerve is unbranched macroscopically for a considerable distance (up to 7 cm.), whereas the N.G.M. is considerably shorter (about 4 cm.) and branches at a level about 1 cm. proximal to its point of entry into the muscle belly. Since the latter nerve has been examined in the rabbit by a number of previous workers, data obtained from it in the present study can be compared directly with figures already published.

METHODS

The specimens obtained at biopsy were gently stretched over cardboard frames to which their ends were made to adhere by gentle pressure. Fixation (24–48 hr.) in Flemming's solution (1 % chromic acid, 15 ml., 2 % osmic acid, 4 ml., glacial acetic acid, 1 drop) was followed by dehydration in ascending concentrations of alcohol and then by paraffin wax embedding. Sample transverse sections (7μ thick) were taken at regular intervals along the nerve and these were placed on glass slides and mordanted in a 3 % solution of potassium bichromate for 6–12 hr. at 37°C . Staining by Kultschitsky's haematoxylin for 24–48 hr. was followed by a second immersion in a 3 % solution of potassium bichromate for 5 min. Differentiation was performed as often as necessary in a solution of 0.25 % potassium permanganate for periods not in excess of 10 sec., after which the slides were placed in Pal's solution for 5 min. Routine dehydration, clearing and mounting were then undertaken.

Measurements of the lengths of the specimens dissected free *in situ* and estimates of their lengths when sectioned and mounted showed that a mean longitudinal shrinkage of 20.5 % had occurred during the above procedures. A considerable proportion of this shrinkage appeared to take place when specimens were cut into two or more segments to facilitate mounting on to cardboard frames of convenient length. Some unavoidable drying was noted during this manoeuvre.

Careful selection of sections suitable for photomicrography ($\times 750$) enabled a series of composite photographs to be collected which was representative of the myelinated fibre populations at many different levels in each of the nerves studied. The absolute magnifications achieved at twenty-eight different photomicrographic sessions varied between 751 and 770 (mean 757.6 ± 0.9). The actual mean over-magnification was thus of the order of 1 %, and this was considered not to affect significantly the diameter measurements and the calculations based on an estimated magnification of $\times 750$.

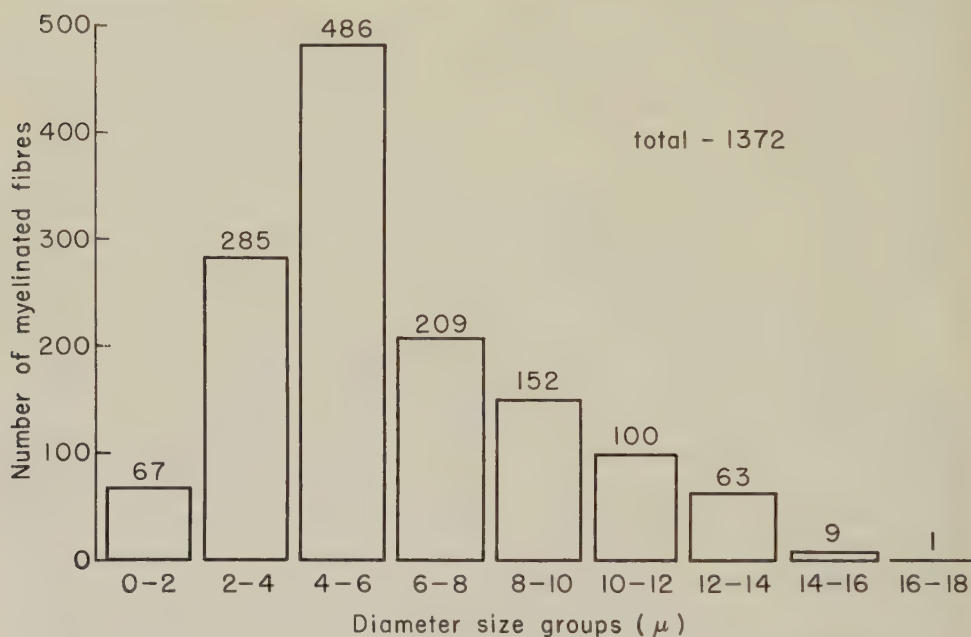
The photographic images of the myelinated fibres were allocated on a basis of their total diameter (i.e. axoplasm plus myelin sheath) to 2μ size groups. This was done by comparison with circles (etched on a transparent Perspex plate) whose diameters corresponded to the maximum diameters of each fibre size group, taking into account the standard magnification factor. As each fibre image was appraised, the sharp point of an actuator connected to an electric counting machine was passed through a hole in the centre of the appropriate etched circle and the fibre image marked by piercing in order to avoid duplicate counting. Examination of several identical photomicrographs of the same section on different occasions revealed that the total numbers of fibres recorded never differed from one another by more than 3.5 %, and that the fibre diameter size frequency distributions obtained were constant as regards the size of the smallest and largest fibre diameter group represented, and the location of the modal diameter size group. However, some variation was apparent in the numbers of the fibres placed within individual size groups. Presumably the allocation between two neighbouring diameter size groups of those fibres, which were of a borderline diameter, was a random phenomenon because the general character of the distribution curves remained closely similar.

Histograms of the fibre diameter size frequency distributions in each section studied were made and the total cross-sectional area of all the myelinated fibres found in each was also calculated.

RESULTS

In a series of eleven non-fasciculated sural nerves from seven adult rabbits it was found that the mean value of the total numbers of myelinated fibres was 1661 ± 83 , and that these fibres varied from less than 2 to 18μ in diameter. The single mode of the fibre diameter size frequency distributions lay between 2 and 6μ (see Text-fig. 1).

Specimen No. II. Omm.



Text-fig. 1. A typical myelinated fibre diameter size frequency distribution in a sural nerve of a rabbit.

Myelinated fibre branches were not identified at any level in the specimens, which varied in length from 15 to 68 mm. Thus in any particular nerve the total number of myelinated fibres found in a section taken at any level was virtually constant. For example, in specimen no. 9 (see Table 1) the total number of fibres was 1372 in the most proximal section available (i.e. 0 mm.). This corresponded to a plane near the great trochanter of the femur, whilst in a section located 68 mm. further distally—in the mid-tibial region—the total recorded was 1370. At two intermediate levels (i.e. 34 and 40 mm.) the corresponding figures were 1384 and 1385 respectively. In no one specimen did the greatest total number of fibres counted exceed the least total by more than 3.5 %. Since this figure was equal to that already found to be the likely limit of experimental error (see methods) this difference was evidently non-significant. Additional support was given to this view by the fact that these discrepancies were not systematic in their arrangement from specimen to specimen and that they

were of a similar order to those implicit in the work of Schiller (1889), Gutmann & Sanders (1943) and Aitken, Sharman & Young (1947).

Tapering of the myelinated fibres was not detected in this series of nerves. Thus the fibre diameter size frequency distribution curves obtained from sections situated at all levels along a particular nerve showed similar limits, shape and mode. Since it was believed that small changes in diameter of the large fibres would be easier to detect than similar changes in small fibres, special attention was paid to the former. It was found that the mean proportion of fibres of more than 10μ in diameter was $12.8\% \pm 0.6$. The number of fibres in the largest size group represented in each nerve never constituted more than 0.5 % of the total number of fibres whilst the number of fibres less than 2μ in diameter never exceeded 2.5 % of the total. Specimen no. 9 (see Table 1) was particularly interesting from this point of view because in the four sections examined, the number of fibres in the 0–2 μ diameter size group

Table 1. *Fibre diameter size frequency distribution in a non-fasciculated sural nerve (rabbit, specimen no. 9)*

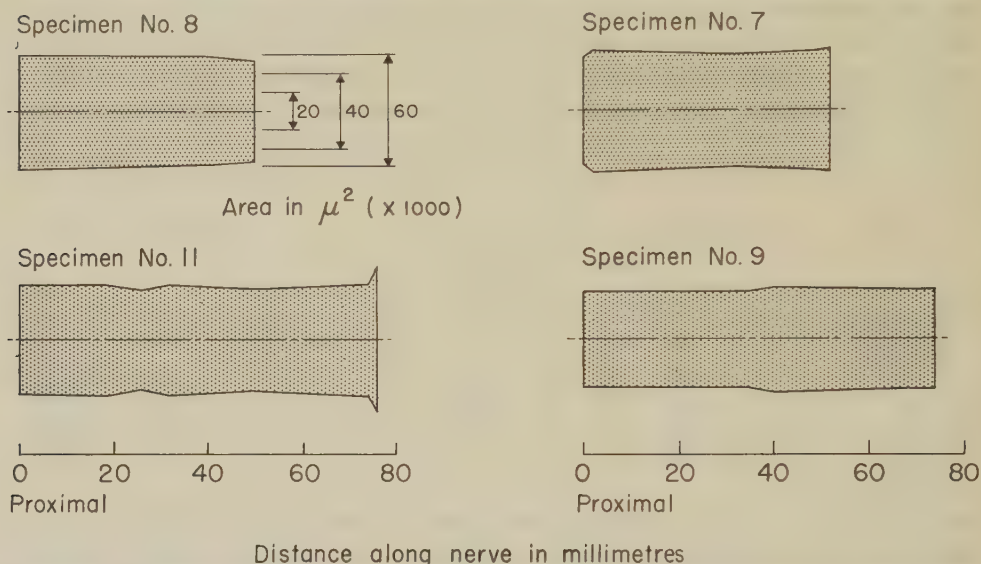
Section level (mm.)	Numbers of myelinated fibres in each diameter size group (μ)									Total number	Total area (μ^2)
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18		
Proximal											
0	67	285	486	209	152	100	63	9	1	1372	49,163
34	65	306	482	180	183	109	53	6	0	1384	48,701
40	72	252	452	167	256	127	54	5	0	1385	53,546
68	61	261	484	161	234	114	50	5	0	1370	50,833
Distal											

lay between 61 and 72, and that in the modal size group (i.e. 4–6 μ) between 452 and 486. The largest fibres (with one exception) were located in the 14–16 μ size group and their number varied from 5 to 9. The fact that a single fibre lay in the 16–18 μ group was not considered to be of significance from the point of view of generalized as opposed to localized fibre tapering since similar inconsistencies were occasionally encountered in other specimens, and the level at which they appeared was inconstant.

In an attempt to verify the absence of myelinated fibre tapering in these nerves, the total cross-sectional area of all the myelinated fibres in each of the sections studied was calculated (see Text-fig. 2). In this series of nerves the mean value of these total cross-sectional areas was $61,887\mu^2 \pm 2864$ whilst the average cross-sectional area of the fibres varied from $30.6\mu^2$ to $42.0\mu^2$ in the same specimens. In specimen no. 9 it was found that at 0, 34, 40 and 68 mm. levels the total cross-sectional areas of all the myelinated fibres were 49,163, 48,701, 53,546, and 50,833 μ^2 and their average cross-sectional areas were 35.8, 35.2, 38.7 and 37.1 μ^2 respectively. In no one non-fasciculated nerve did the greatest total cross-sectional area calculated exceed the least total cross-sectional area by more than 14 %, and as these apparent discrepancies were not systematically arranged they were believed to give an indication only of the accuracy of the diameter measurements. Since the calculation of these areas involved squaring of the radii, any inaccuracies made in the fibre diameter measurements were disproportionally magnified. Bearing this in mind, it became evident that the maximum experimental error occurring in making a series of diameter measurements was very little greater than that involved in counting

fibres. Thus the maximum experimental counting error was 3.5 % and this, when squared, gave a value of 12.25 % which was comparable with the maximum recorded deviation (i.e. 14 %) in the area calculations from level to level in any one specimen.

Myelinated branches were identified in specimen no. 11 (see Table 2) distal to the plane at which the hitherto non-fasciculated trunk split up into several microscopic bundles (i.e. level 71 mm.). Thus, whereas only 1448 fibres were counted in a section taken 20 mm. proximal to this level (i.e. 50 mm.), in another section taken 5 mm.



Text-fig. 2. A schematic representation of the total cross-sectional area of the myelinated fibres at various levels in four typical sural nerves of rabbits. (Fibre branching distally in specimen no. 11 accounts for the enlargement of this figure towards the right.)

Table 2. *Fibre diameter size frequency distribution in a fasciculated sural nerve (rabbit, specimen no. 11)*

Section level (mm.)	Numbers of myelinated fibres in each diameter size group (μ)									Total number	Total area (μ^2)
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18		
Proximal											
50	66	185	606	184	237	114	48	8	—	1448	53,859
71				Fasciculation starts here							
74	72	264	562	141	173	156	73	28	—	1469	58,852
76	51	232	533	232	211	178	141	35	—	1613	76,813
Distal											

distal to the point of fasciculation (i.e. level 76 mm.) 1613 fibres were counted. This increase of 165 fibres represented an augmentation of 11.4 %, and was at least three times the maximum estimated experimental error. At first this was taken to indicate that about one in ten of the fibres dichotomized near the site of fasciculation. However, of perhaps even greater interest were the differences which became apparent when the fibre diameter size frequency distributions at the two levels were compared. Although their limits and the location of the modal group remained unchanged,

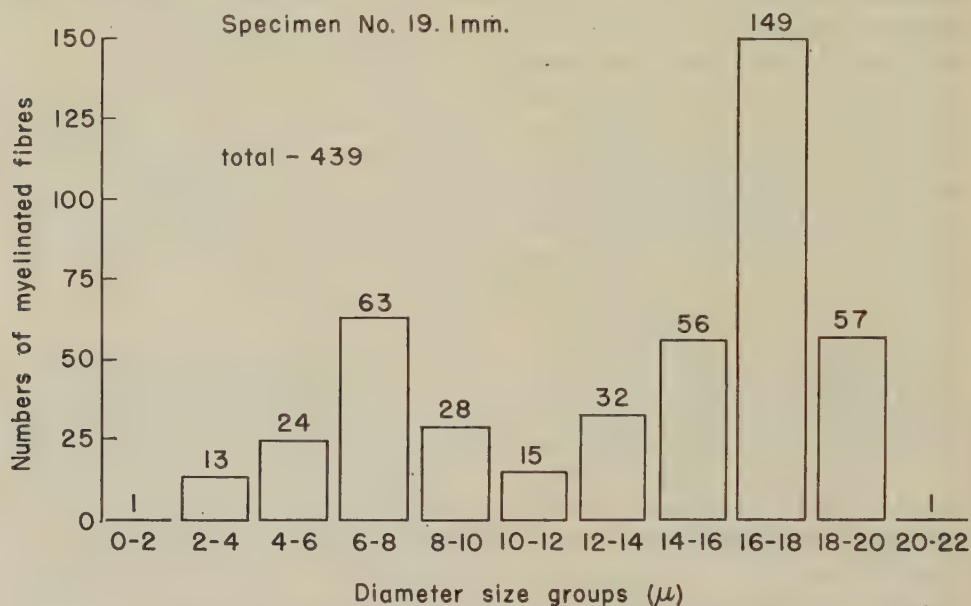
it could be readily seen that there was a marked increase from the proximal to the distal level in the number of fibres exceeding 10μ in diameter. This accounted for the whole of the increase in the total numbers of fibres between the two levels, and at first suggested that each of the fibres of this calibre had given rise to two myelinated branches. Closer inspection of the fibre representation in the higher diameter size groups indicated that if this were so, each parent fibre must have given rise to one daughter fibre which was larger than, and another daughter fibre which was as large as, the parent in diameter. An alternative, but less likely, interpretation of the data was that roughly one half of the fibres in the 10 – 12μ diameter size group had given rise to two branches each and that both daughter fibres were approximately of the same diameter as their parent. In addition, every parent fibre in the 12 – 14μ diameter size group had produced three branches, each with a diameter equal to that of their parent. By the same reasoning each parent fibre in the 14 – 16μ diameter size group had divided into four branches, each of which possessed a diameter equal to that of their parent.

That enlargement of fibres took place distal to the point of fasciculation, received additional support from the fact that in specimen no. 11 the total cross-sectional area of the myelinated fibres in the non-fasciculated section proximally (i.e. level 50 mm.) was $53,859\mu^2$, and that in the multifasciculated section of the same nerve distally (i.e. level 76 mm.) this value was $76,313\mu^2$ (see Table 2 and Text-fig. 2). This was an increase of 42 %, which was about three times the previously determined maximum experimental error. The average cross-sectional area of the fibres in these two sections increased from $37.2\mu^2$ in the more proximal section to $47.3\mu^2$ in the more distal section.

In a series of eight non-fasciculated nerves to the head of the gastrocnemius muscle (N.G.M.) from five adult rabbits, the total numbers of myelinated fibres lay between 356 and 439 (mean value 408) in sections taken between 2 and 3 cm. proximal to the point of entry of the nerve into the muscle belly. The diameters of the fibres varied from less than 2 to 22μ , and the two modes in the fibre diameter size frequency distributions lay between 4 – 10μ and 14 – 20μ (see Text-fig. 3). The mean value of the proportion of fibres of a diameter in excess of 10μ was 73.5 % and those fibres of less than 2μ in diameter never exceeded 2.5 % of the total. The total cross-sectional area of all the myelinated fibres varied from $54,435$ to $80,690\mu^2$ (mean value $67,909\mu^2$). A standard deviation for both of the mean values quoted was calculated, but because the series of figures from which they were derived were not distributed in a 'normal' manner these statistics proved to be misleading and have therefore not been reported. (A similar difficulty is implicit in the work of Swensson (1949) on the myelinated fibres in the trochlear nerve of rabbits.) The average cross-sectional area of the fibres in the various sections studied varied from 134.7 to $203.8\mu^2$. This figure was about five times that found in the sural nerve, although the mean values of the total cross-sectional areas of all the fibres in both series of nerves were, by chance, somewhat similar.

Over distances of 5 mm. in the non-fasciculated N.G.M. neither myelinated fibre branching nor tapering was detected. Thus in specimen no. 19 the numbers of fibres in the lower and upper modal size groups in sections 5 mm. distant from one another were 63 and 42 and 149 and 148 respectively. Each nerve possessed one fibre in the 20 – 22μ diameter size group which was the largest group represented (see Table 3).

The sex, type (breed) or body weight of an adult animal or the side of the body from which a specimen was taken did not affect the data obtained from either nerve. The first three of these findings were in contrast to the findings of Dunn (1912) and McCrady (1934).



Text-fig. 3. A typical myelinated fibre diameter size frequency distribution in a nerve to the medial head of the gastrocnemius muscle in a rabbit.

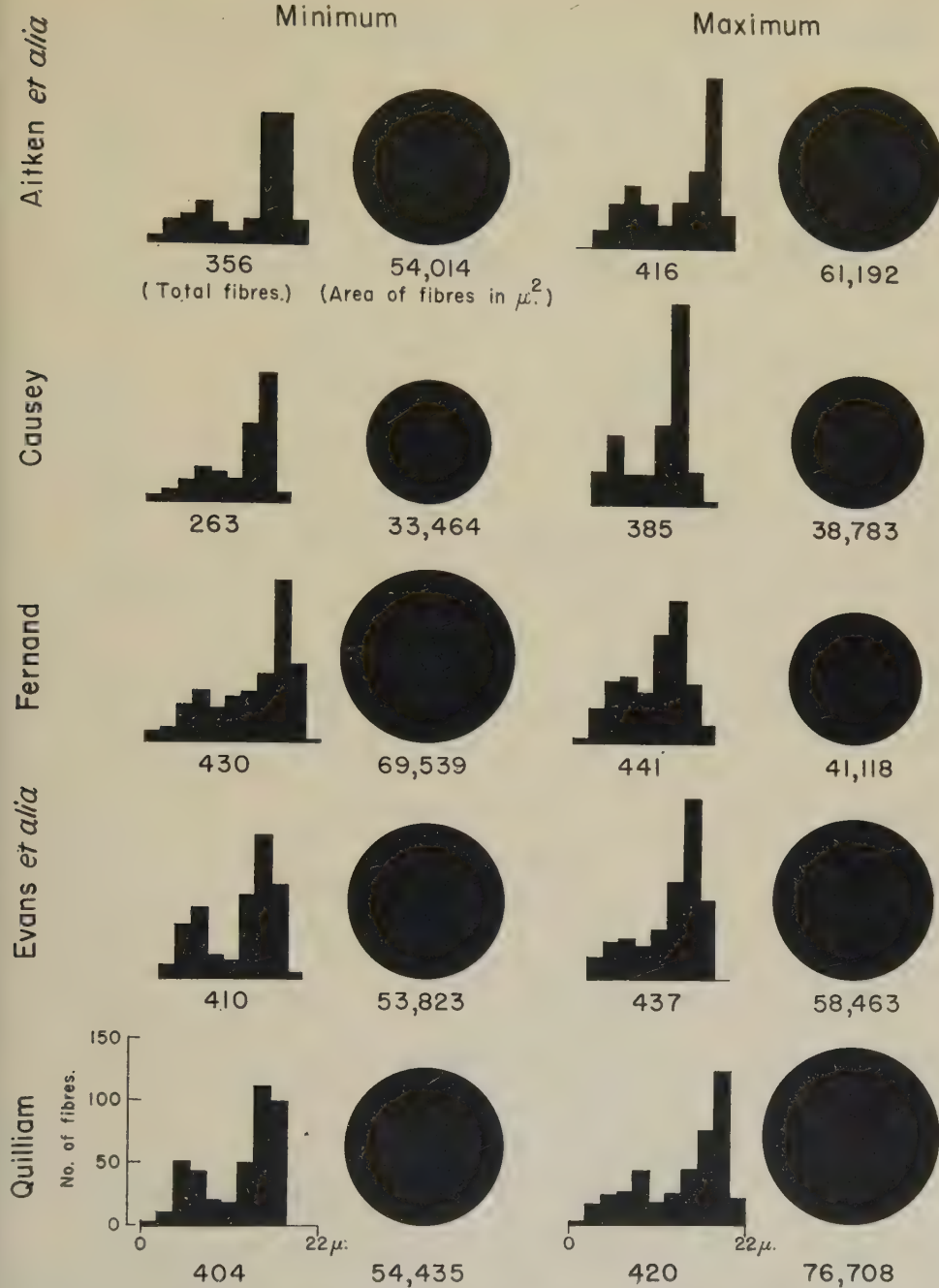
Table 3. *Fibre diameter size frequency distribution in a nerve to the medial head of the gastrocnemius muscle (rabbit, specimen no. 19)*

Section level (mm.)	Numbers of myelinated fibres in each diameter size group (μ)											Total number	Total area (μ^2)
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22		
Proximal													
1	1	13	24	63	28	15	32	56	149	57	1	439	70,666
3	2	9	32	44	29	24	24	66	139	57	2	428	69,766
5	3	15	28	42	19	17	26	77	148	38	1	414	66,871
Distal													

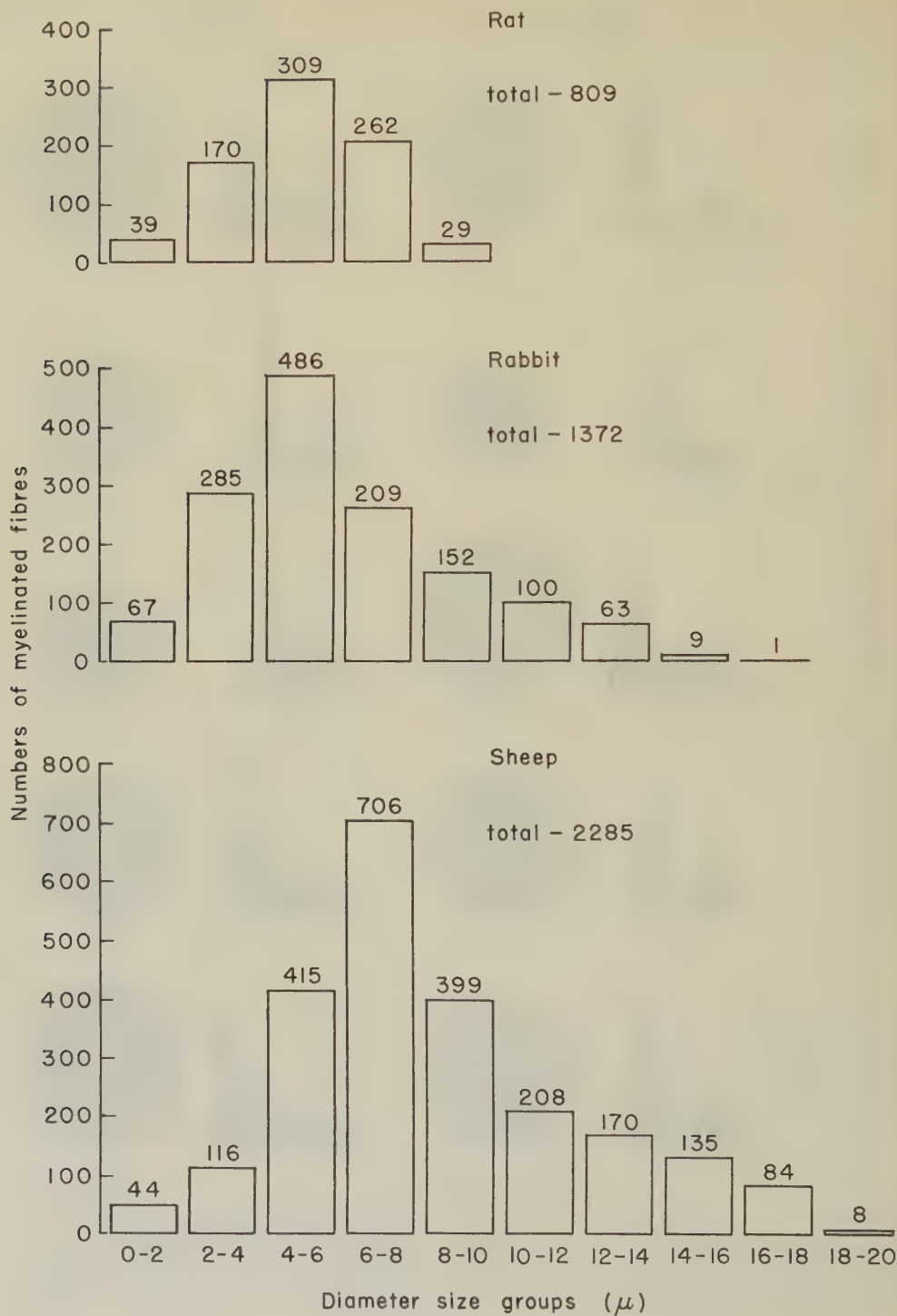
However, the figures quoted were in substantial agreement with those of Aitken *et al.* (1947), Causey (1948), Fernand & Young (1951) and Evans & Vizoso (1951) (see Text-fig. 4). Each of these workers used methods similar to those described here except for minor modifications. For instance, Causey (1948) used fixation *in situ* and Evans & Vizoso (1951) used a photographic magnification of $\times 1000$.

It is thus evident that direct comparisons can be made between data obtained from different nerves by various authors, providing the methods used are similar to those employed in the present study.

In view of Duncan's (1933) contention and Häggqvist's (1948) denial that corresponding nerve trunks in bulky animals possessed more and larger myelinated fibres than those in lighter animals, it was decided to study the myelinated fibres in the sural nerve and the N.G.M. of a sheep (body weight *c.* 150 lb.), which was 30 times

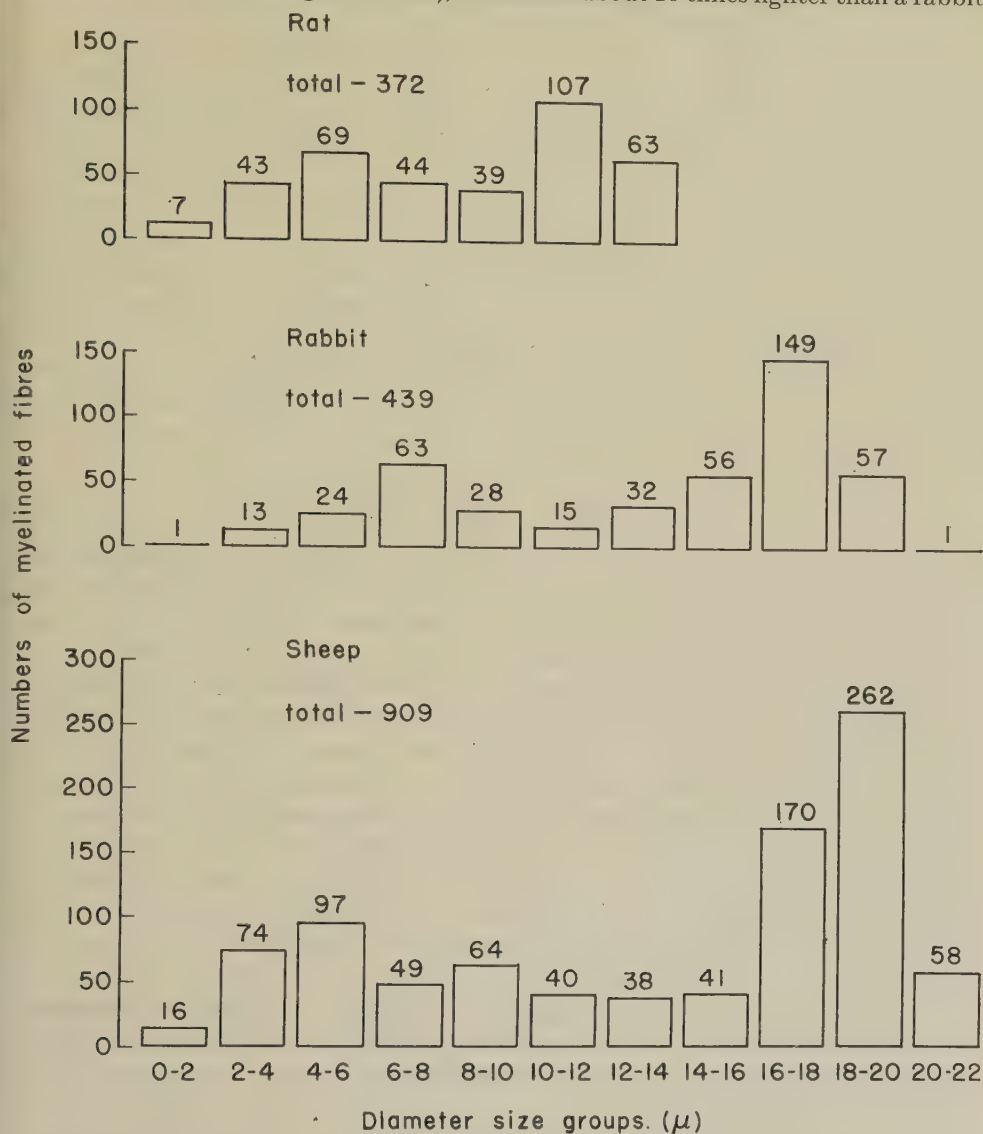


Text-fig. 4. A comparison between the fibre diameter size frequency distributions and the total cross-sectional areas of the myelinated fibres in series of nerves to the medial head of the gastrocnemius muscle in rabbits as reported by various authors. [Those specimens possessing the most and the fewest fibres in each series are represented.]



Text-fig. 5. Typical myelinated fibre diameter size frequency distributions in the sural nerves of a rat, a rabbit and a sheep.

heavier than a rabbit (body weight *c.* 5 lb.); and also to examine the fibres in these nerves in a rat (body weight *c.* 10 oz.), which was about 10 times lighter than a rabbit.



Text-fig. 6. Typical myelinated fibre diameter size frequency distributions in the nerves to the medial head of the gastrocnemius muscle in a rat, a rabbit and a sheep.

When the sural nerves were considered (see Text-fig. 5) it was found that there were many more fibres in the nerve taken from the sheep (*i.e.* 2285) than in a typical example (specimen no. 9) from the rabbits (*i.e.* 1372), which in turn possessed nearly twice as many fibres as that from the rat (*i.e.* 809). The mode of the fibre diameter size frequency distribution lay in the 6-8 μ group in the sheep, and in the 4-6 μ group in the rabbit and rat. In the sheep, 26.4% of the fibres exceeded 10 μ in diameter,

in the rabbit this proportion was 12.7 % and in the rat there were no fibres greater than 10μ in diameter. In the sheep ninety-two fibres exceeded 16μ in diameter, whereas only one fibre did so in the rabbit.

When the N.G.M.'s from these animals were compared (see Text-fig. 6) it was found that the nerve from the sheep possessed more than twice as many fibres (i.e. 909) as that in a typical example (specimen no. 19) from the rabbits (i.e. 439), and that from the rat (i.e. 372). The fibre diameter size frequency distributions were bimodal in character in each case. In the nerves from the sheep and the rat, the lower mode lay in the $4-6\mu$ size group, whilst in that from the rabbit it was located in the $6-8\mu$ group. In the sheep, the upper mode was found in the $18-20\mu$ group, in the rabbit in the $16-18\mu$ group, and in the rat in the $10-12\mu$ group. 66.8 % of the fibres in the sheep, 71.2 % in the rabbit, and 45.7 % in the rat exceeded 10μ in diameter. Fifty-eight fibres exceeded 20μ in diameter in the sheep but only one did so in the rabbit, whilst the largest fibres in the rat were 14μ in diameter. The proportion of fibres of less than 4μ in diameter was 7.0, 25.7 and 24.5 % in the sural nerves and 10.0, 3.2 and 13.5 % in the N.G.M.'s of the sheep, rabbit and rat, respectively.

DISCUSSION

The measurement of diameters in aggregations of myelinated nerve fibres presents a number of problems which are not entirely overcome by substituting allocation of the fibres to specified arbitrary size groups for individual determination of absolute values.

The occurrence of fibres that are not truly circular in outline is a difficulty not easily overcome. If an estimate be made of their diameter, had they been circular, then the validity of this process is clearly inversely proportional to the degree of irregularity of the fibre outline. When the myelin is indented, the fibre is said to be crenated and considerable error in estimating its diameter may occur. Duncan (1934) and Aitken *et al.* (1947) maintained that crenation was due to shrinkage occurring as a result of inadequate fixation and placed such a fibre in a size group one higher than it would otherwise seem to occupy. Later, Hess & Young (1952) showed that there were irregularities of fibre outline for 15 or 20μ on either side of a node of Ranvier and that spiral corrugations on the inner surface of the myelin sheath were present even further afield.

This observation explained the apparent anomaly of crenated fibres occurring next to fibres which were perfectly circular in outline in certain transverse sections. Thus crenation appears to be a sign of inadequate fixation only if it is comparatively widespread throughout a fasciculus.

Using the fibre diameter/internode ratio proposed by Vizoso & Young (1948) it is possible to estimate the mean incidence of crenated fibres in well-fixed specimens. Thus in the case of the N.G.M. of rabbits, the theoretical calculation suggests that from twelve to fifteen fibres should be crenated and that two to five nodes of Ranvier should be present in each section. These expectations are largely realized in practice, but the proportion of fibres concerned is too small to significantly affect the fibre diameter size frequency distributions. A number of authors have noted that individual fibres are subject to considerable variation in diameter from level to level (e.g. Eccles & Sherrington, 1930; Hess & Young, 1952; Quilliam & Sato, 1955). In

view of the lack of consistently uniform fibre diameters and because of the experimental errors already mentioned, minor deviations in the shape of a fibre diameter size frequency distribution from level to level along a particular nerve are not necessarily indicative of any real alteration in the fibre population. Before significance can be attached to it, a change in the fibre diameter size frequency distributions should be well marked and several times the limit of the known experimental error or, if smaller than this, then it must be regularly demonstrable and be systematically arranged.

No matter how accurate the measurement in a stained section, the pertinent question remains—what is the relation of the data obtained to the fibre diameter size frequency distribution *in vivo*? This subject has been studied by, among others, Sherrington, (1894), Duncan (1934), Arnell (1936), Taylor (1942), Rexed (1944). It is generally agreed that of all the available myelin stains those involving the use of osmic acid shrink the fibres least and that this loss of calibre does not exceed 10 % of the original value.

In an endeavour to typify the size characteristics of fibres in particular nerve trunks for comparative purposes, various authors have calculated the factors known as the 'root mean square' (or *D*) or the 'average mean diameter' (or A.M.D.) from fibre diameter size frequency distributions. Whereas these factors are useful when unimodal fibre diameter size frequency distributions are being characterized they are apt to prove unreal when the distributions being considered are bimodal in character since the value for *D* or A.M.D. may fall in the sparsely populated trough between the two peaks. The total cross-sectional area of all the myelinated fibres within a section, on the other hand, is an actual measure of a physical property of the fibre population. In addition, by the application of appropriate ratios (e.g. axon/myelin ratio of the fibres or fibre/connective tissue ratio of the nerve trunk) further useful information can be derived from it. In practice this value remains remarkably constant (to within 14 %) from level to level within the same intact nerve in spite of the fact that any errors in the diameter measurements from which it is derived are squared. The variation manifest from specimen to specimen is susceptible to statistical evaluation much in the same way as is that in the total numbers of fibres. The average cross-sectional area of the fibres in a section is another useful figure which can be employed when the 'typical' size of the fibres within two different fibre populations are being compared.

The sural nerve is one of the comparatively few nerves studied to date whose fibre diameter size frequency distribution is unimodal in type (Quilliam, 1950). Unimodality does not seem to be characteristic of all cutaneous nerves (Davenport, Droegemuller, Fisher & Ranson, 1934). On the other hand, this type of distribution is also found in many spinal roots both dorsal and ventral (Rexed, 1944) and in certain nerves passing to muscles having few (if any) spindles (see Fernand & Young, 1951). However, unimodality is found in certain cutaneous branches of mixed nerves (Sanders & Young, 1944), in the ventral caudal nerve of the tail of the rat (Chatfield & Lyman, 1954) and in the dorsal nerve to the penis in the steer (Quilliam, 1955) but not in human sural nerves (Tomasch & Schwarzscher, 1952).

Distally directed tapering of myelinated nerve fibres in mammals has long been suspected since it has been shown to occur in the giant fibres of cephalopods (Young,

1939) and at the sites of dichotomy in peripheral nerves in cats (Eccles & Sherrington, 1930). The data published by Swensson (1944) and Causey (1948) suggest the absence of detectable tapering in the myelinated fibres of the trochlear and abducens nerve on the one hand and of the N.G.M. of the rabbit on the other but the distances available for study were somewhat restricted. Rexed (1944), using the human phrenic nerve, was of the opinion that myelinated fibre tapering did not occur over considerable distances without concomitant fibre branching. The present work is of especial interest because it shows that in the non-fasciculated segment of the sural nerve about 7 cm. long, which lies approximately midway between the cells of origin of its fibres in the dorsal root ganglia and their cutaneous end organs, branching and tapering of its myelinated fibres cannot be detected.

Fasciculation of nerve trunks distally has hitherto been little studied. It is presumably the precursor of macroscopic branching, and the present work suggests that it is a site of myelinated fibre branching and the site of fibre enlargement. However, examination of serial sections or longitudinal sections must be made before the quantitative aspects of this type of branching can be fully determined. Cooper (1929), using teased specimens from the nerve trunk to the sartorius muscle in a cat, showed that myelinated nerve fibres sometimes split into two or even three branches at a level about 1 cm. proximal to the point of entry of the nerve into its muscle. The work of Lavarack, Sunderland & Ray (1949, 1951) on the human sural nerve supports the present finding that when a myelinated fibre divides, one of its branches may possess a diameter in excess of that of the parent proximally.

Duncan (1933) measured the diameters of the myelinated fibres in the dorsal spinal roots of the cow, rat and cat and found that the largest fibres they contained were 23, 16 and 15 μ respectively. The percentage of fibres of less than 2.6 μ in diameter in the cow (23 %) was between two and three times as great as that in the rat and the cat (9 and 7 %). These figures prompted him to make the suggestion that during development, more of the larger unmyelinated fibres in the cow than in the smaller animals came to exceed the critical threshold diameter (1–2 μ) at which myelination 'automatically' occurred. Also, enlargement during normal growth of the myelinated fibres of all sizes in the cow was greater than in the smaller animals. However, Rexed (1944) showed that in the dorsal spinal root of the first sacral nerve the largest fibres present in the rabbit were 13 μ in diameter, in the dog 18 μ , and in man 14 μ . Yet, in the corresponding ventral spinal root the largest fibres in the rabbit and cat were 15 μ and in dog and man 18 μ respectively.

That the maximum size of myelinated fibres in a specific peripheral nerve is to some extent determined by the bulk of the adult animal from which the specimen is removed receives partial confirmation in the present work when animals of different species are concerned. However, this does not seem to be the case when individuals of the same species but of different body weights are concerned.

SUMMARY

1. A study has been made of the total numbers and diameter size frequency distributions of the myelinated fibres present in transverse sections of sural nerves and nerves to the medial head of the gastrocnemius muscle (N.G.M.) in a series of rabbits, and also in a rat and a sheep.

2. In a series of eleven sural nerves from rabbits, the mean value of the total numbers of fibres recorded was 1661 ± 83 , and the mean value of the total cross-sectional areas of the fibres was $61,887 \mu^2 (\pm 2864)$.

3. In eight N.G.M.'s the mean total number of myelinated fibres was 408 and the mean value of the total cross-sectional area of the fibres was $67,909 \mu^2$.

4. In all nerves studied, myelinated fibres of less than 2μ in diameter were found.

5. In rabbits the largest fibres present in the sural nerves were 18μ in diameter and in the N.G.M. 22μ in diameter. In the former nerve, the fibre diameter size frequency distributions were unimodal, the peak being found in either the $2-4 \mu$ or the $4-6 \mu$ group. In the latter nerve there were two peaks in the size distributions, the lower one lying between 4 and 10μ and the upper between 14 and 20μ . The mean proportions of the total numbers of fibres of more than 10μ in diameter were 12.8 % in the sural nerves and 73.5 % in N.G.M.'s.

6. In rabbits, branching and tapering of the myelinated fibres was not detected in the non-fasciculated sural nerves of rabbits over distances of up to 68 mm. or in the non-fasciculated N.G.M.'s over distances of up to 5 mm.

7. Distal fasciculation of the sural nerve in the rabbit was accompanied by the production of myelinated fibre branches. Certain of these appeared to be larger in diameter than their parent fibre.

8. In the sural nerve from a sheep there were 2285 myelinated fibres. The single mode of the diameter size frequency distribution lay in the $6-8 \mu$ group. No fibre exceeded 20μ in diameter. 26.4 % of the fibres exceeded 10μ in diameter.

9. In the sural nerve of the rat, there were 809 fibres. The single mode of the fibre diameter size frequency distribution was found in the $4-6 \mu$ diameter size group. No fibre exceeded 10μ in diameter.

10. In the N.G.M. of the sheep there were 909 fibres. The lower mode of the diameter size frequency distribution lay in the $4-6 \mu$ group and the upper in the $18-20 \mu$ group. No fibre exceeded 22μ in diameter. 66.8 % of the fibres exceeded 10μ in diameter.

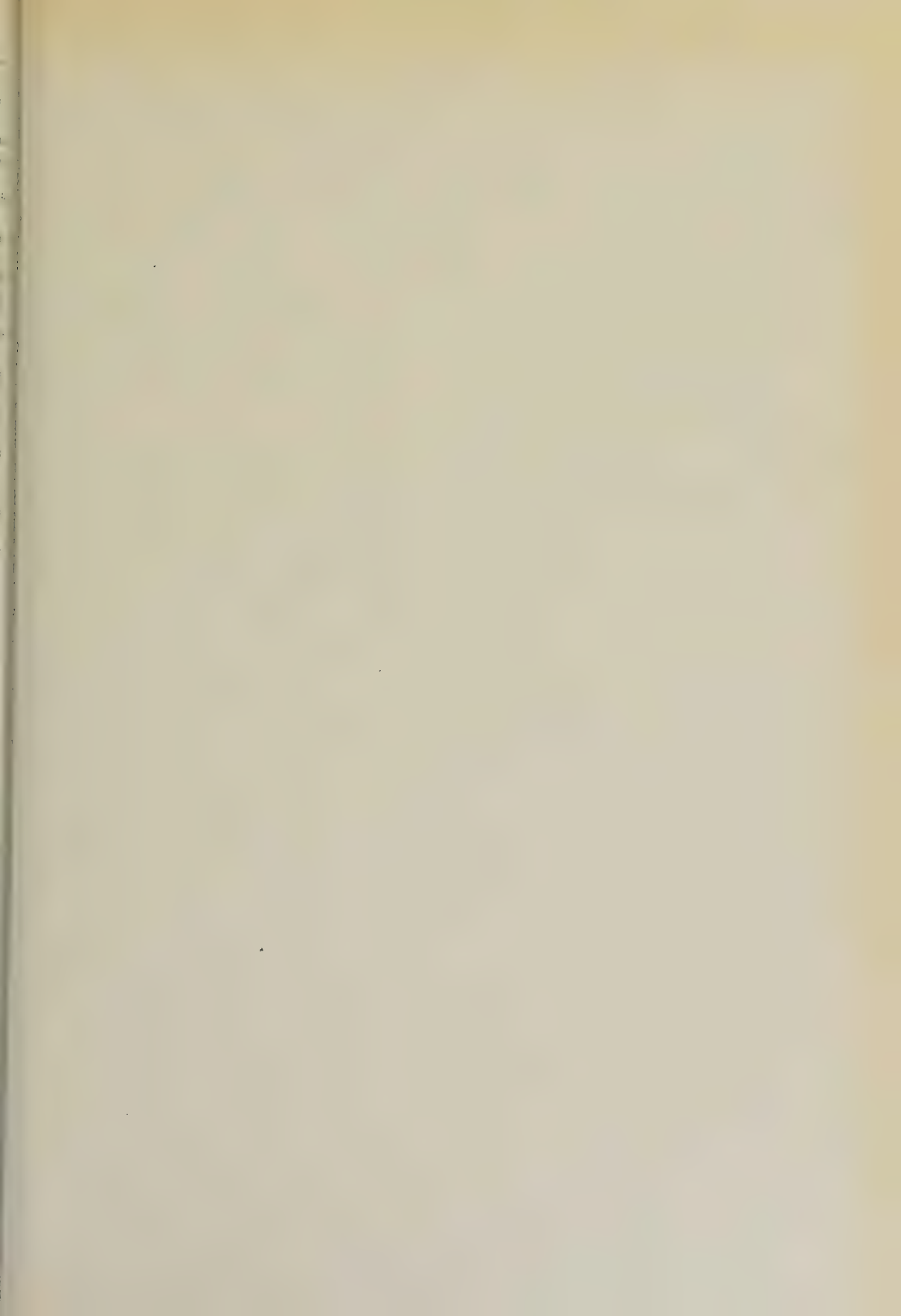
11. In the N.G.M. of the rat there were 372 fibres. The lower mode of the diameter size frequency distribution lay in the $4-6 \mu$ group, and the upper mode in the $10-12 \mu$ group. No fibre exceeded 14μ in diameter. 45.7 % of the fibres exceeded 10μ in diameter.

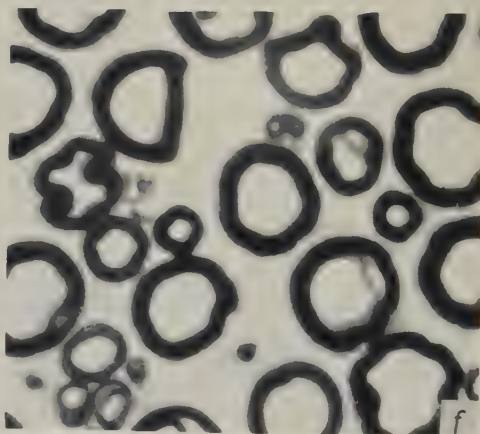
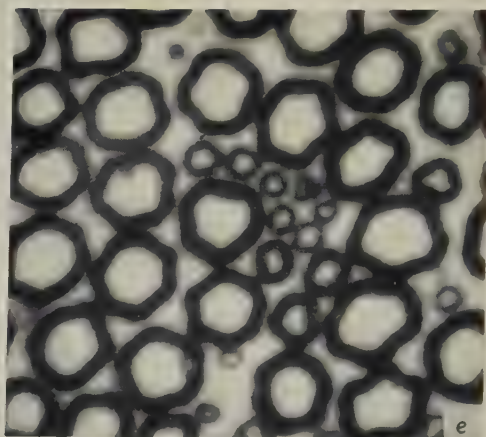
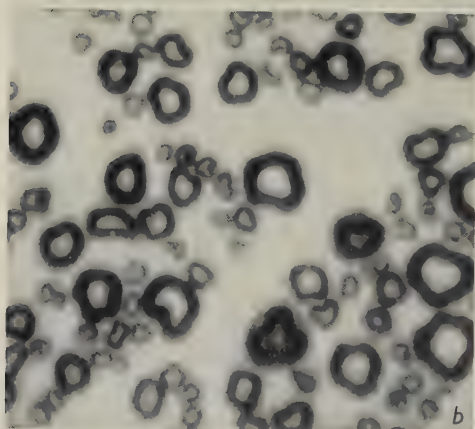
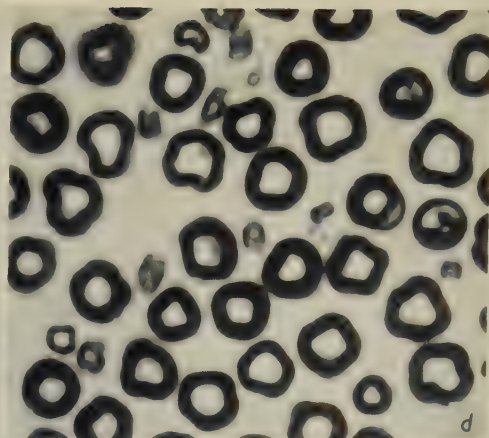
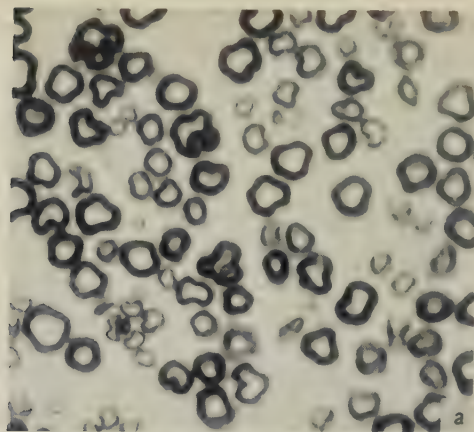
I wish to thank Prof. J. Z. Young for his help and encouragement during this study. I am also indebted to Mr D. A. Sholl for guidance in the use of statistical methods and to Mr J. Armstrong and his technical staff for their assistance. Material from the sheep was obtained through the courtesy of Dr Ralph Kitchell of the Division of Veterinary Anatomy, University of Minnesota.

REFERENCES

- AITKEN, J. T., SHARMAN, M. & YOUNG, J. Z. (1947). Maturation of regenerating nerve fibres with various peripheral connexions. *J. Anat., Lond.*, **81**, 1-22.
ARNELL, N. (1936). Untersuchung über die Dicke des Achsenzylinders und der Markscheide in nicht fixierten Spinalnerven des Menschen. *Acta psychiat., Kbh.*, **11**, 5-25.
CAUSEY, G. (1948). The effect of pressure on nerve fibres. *J. Anat., Lond.*, **82**, 262-270.

- CHATFIELD, P. O. & LYMAN, C. P. (1954). The effects of temperature on the ventral caudal nerve of the rat. *Amer. J. Physiol.* **177**, 183-186.
- COOPER, S. (1929). The relation of active to inactive fibres in fractional contraction of muscle. *J. Physiol.* **67**, 1-13.
- DAVENPORT, H. K., DROEGEMULLER, W. H., FISHER, C. & RANSON, S. W. (1934). Number, size and myelination of the sensory fibers in the cerebrospinal nerves. *Res. Publ. Ass. nerv. ment. Dis.* **15**, 3-34.
- DUNCAN, D. (1933). The diameters of dorsal root fibres in some common mammals. *Anat. Rec.* **55** (Suppl.), 55.
- DUNCAN, D. (1934). A relation between axone diameter and myelination determined by measurement of myelinated spinal root fibres. *J. comp. Neurol.* **60**, 437-462.
- DUNN, E. H. (1912). The influence of age, sex, weight and relationship upon the number of medullated nerve fibres in the ventral root of the second cervical nerve of the albino rat. *J. comp. Neurol.* **22**, 131-156.
- ECCLES, J. C. & SHERRINGTON, C. S. (1930). Numbers and contraction values of individual motor units examined in some muscles of the limb. *Proc. Roy. Soc. B*, **106**, 326-357.
- ERLANGER, J. & GASSER, H. S. (1937). *Electrical Signs of Nervous Activity*. Philadelphia: University of Pennsylvania.
- EVANS, D. H. L. & VIZOSO, A. D. (1951). Observations on the mode of growth of motor fibres in rabbits during post-natal development. *J. comp. Neurol.* **95**, 429-462.
- FERNAND, V. S. V. & YOUNG, J. Z. (1951). The sizes of the nerve fibres of muscle nerves. *Proc. Roy. Soc. B*, **139**, 38-58.
- FONTANA, F. (1781). *Traité sur le venin de la vipère, etc.* **2**, p. 40. Florence.
- GASKELL, W. H. (1886). The structure, distribution and function of the nerves which innervate the visceral and vascular system. *J. Physiol.* **7**, 1-80.
- GUTMANN, E. & SANDERS, F. K. (1943). Recovery of fibre numbers and diameter in the regeneration of peripheral nerves. *J. Physiol.* **101**, 489-518.
- HÄGGQVIST, G. (1948). Nervenfaserkaliber beim Tieren verschiedener Größe. *Anat. Anz.* **96**, 398-412.
- HESS, A. & YOUNG, J. Z. (1952). The nodes of Ranvier. *Proc. Roy. Soc. B*, **140**, 301-320.
- HURSH, J. B. (1939). Conduction velocity and diameter of nerve fibres. *Amer. J. Physiol.* **127**, 131-139.
- KUFFLER, S. W., HUNT, C. C. & QUILLIAM J. P. (1951). Mammalian small nerve-fibres. *J. Neurophysiol.* **14**, 29-54.
- KUFFLER, S. W., LAPORTE, Y. & RANSMEIER, R. E. (1947). The function of the frog's small-nerve motor system. *J. Neurophysiol.* **10**, 395-408.
- LAVARACK, J. O., SUNDERLAND, S. & RAY, L. J. (1949). The caliber of nerve fibres in human cutaneous nerves. *J. comp. Neurol.* **91**, 87-101.
- LAVARACK, J. O., SUNDERLAND, S. & RAY, L. J. (1951). The branching of nerve fibres in human cutaneous nerves. *J. comp. Neurol.* **94**, 293-311.
- MCCRADY, E., JR. (1934). The motor nerves of the eye in albino and Norwegian grey rats. *J. comp. Neurol.* **59**, 285-300.
- MONRO, A. Quoted by DUNCAN, A. (1779). *Medical and Philosophical Commentaries of the Medical Society of Edinburgh, London*, **6**, 111-113.
- MONRO, A. (1783). *Observations on the Structure and Functions of the Nervous System*. Edinburgh: W. Creech.
- QUILLIAM, T. A. (1950). Diameter variations of nerve fibres along normal and regenerating sensory nerve trunks. *Proc. 5th Int. Anat. Congr., Oxford*, **1**, 156-157.
- QUILLIAM, T. A. (1955). The myelinated fibre diameter size frequency distribution in the dorsal nerve to the penis of the steer. *Anat. Rec.* **122**, 661.
- QUILLIAM, T. A. & SATO, M. (1955). The distribution of myelin on nerve fibres from Pacinian corpuscles. *J. Physiol.* **129**, 167-176.
- REXED, B. (1944). The post-natal development of the peripheral nervous system in man. *Acta psychiat., Kbh.* (Suppl.), **33**.
- SANDERS, F. K. & YOUNG, J. Z. (1944). The role of the peripheral stump in the control of fibre diameter in regenerating nerves. *J. Physiol.* **103**, 119-136.
- SCHILLER, M. H. (1889). Sur le nombre et le calibre des fibres nerveuses du nerf oculomoteur commun, chez le chat nouveau-né et chez le chat adulte. *C.R. Acad. Sci., Paris*, **109**, 530-532.





QUILLIAM — SOME CHARACTERISTICS OF MYELINATED FIBRE POPULATIONS

(Facing p. 187)

- SCHWALBE, G. (1882). *Über die Kaliberverhältnisse der Nervenfasern*. Leipzig.
- SHERINGTON, C. S. (1894). On the anatomical constitution of nerves of skeletal muscles. *J. Physiol.* **17**, 211–258.
- SWENSSON, A. (1944). Cited by REXED, B. (1944). The postnatal development of the peripheral nervous system in man. *Acta psychiat., Kbh.* (Suppl.), **33**, 111.
- SWENSSON, A. (1949). Faseranalytische Untersuchungen am Nervus trochlearis und Nervus abducens. *Acta anat.* **7**, 154–172.
- TASAKI, I., MARUHASHI, J. & MIZUGUCHI K. (1952). Action currents in single afferent nerve fibres elicited by stimulation of the skin of the toad and the cat. *J. Physiol.* **117**, 129–151.
- TAYLOR, G. W. (1942). Optical properties of cat nerve fibres. *J. cell. comp. Physiol.* **20**, 359–372.
- TOMASCH, VON J. & SCHWARZACHER, H. G. (1952). Die innere Struktur peripherer menschlicher Nerven im Lichte faseranalytischer Untersuchungen. *Acta anat.* **16**, 315–354.
- VIZOSO, A. D. & YOUNG, J. Z. (1948). Internode length and fibre diameter in developing and regenerating nerves. *J. Anat., Lond.*, **82**, 110–134.
- YOUNG, J. Z. (1939). The structure of nerve fibres in cephalopods and crustacea. *Proc. Roy. Soc. B*, **121**, 319–337.

EXPLANATION OF PLATE

Microphotographs of transverse sections of (1) the sural nerve in (a) the rat, (b) the rabbit and (c) the sheep; and (2) the nerve to the medial head of the gastrocnemius muscle in (d) the rat, (e) the rabbit and (f) the sheep. (Modified Weigert-Pal stain, $\times 750$.)

A NOTE ON TERMINAL DEGENERATION IN THE HYPOTHALAMUS

BY W. M. COWAN AND T. P. S. POWELL

Department of Anatomy, University of Oxford

During investigations which we have been making on fronto-hypothalamic connexions in the macaque monkey, serious doubts have arisen regarding the validity of the Glees silver technique for demonstrating terminal degeneration in certain areas of the hypothalamus. In the first place, re-examination of experimental material has made it clear that the distribution and severity of terminal degeneration in the hypothalamus and preoptic areas showed no apparent difference in relation to the site and extent of the lesion in the frontal lobe. In each experiment characteristic 'terminal degeneration' was found in the lateral septal nucleus, the nucleus accumbens, the bed nucleus of the stria terminalis, the medial preoptic and anterior hypothalamic areas and the paraventricular hypothalamic nucleus. In the dorsomedial, ventromedial and the periventricular arcuate nuclei the 'terminal degeneration' was found to be particularly severe. Secondly, the fact that in every case the appearance of 'degeneration' was bilaterally symmetrical in its distribution led us to doubt whether it was the result of true degeneration. These doubts were accentuated after an examination of the literature, in which it was found that degeneration had been described in these nuclei following lesions in such widely different systems as the amygdala, frontal neocortex, hippocampus and olfactory bulb. Thus, afferents to the bed nucleus of the stria terminalis have been described as originating from the olfactory bulb (Le Gros Clark & Meyer, 1947; Meyer & Allison, 1949; Adey, 1953), the amygdaloid nuclei (Adey & Meyer, 1952) and all areas of the frontal lobe (Meyer, 1950). Again, particularly severe degeneration has consistently been found in the ventromedial hypothalamic nucleus after lesions involving the frontal lobe (Meyer, 1950; Wall, Glees & Fulton, 1951), the amygdala (Adey & Meyer, 1952) and the hippocampus (Simpson, 1952).

In view of these observations it became necessary to make a critical study of control animals, preferably without an intracranial lesion. For this purpose, sections of the preoptic and hypothalamic areas of a monkey, in which one eye had been removed 7 days before death, were cut and stained by the Glees method. The brain was fixed by vascular perfusion of 10 % formol-saline. The appearance of apparently typical 'terminal degeneration' was found bilaterally in precisely the same areas and, as far as can be judged, of the same severity as has been described in experimental material. This finding led to the study of the brains of three normal macaque monkeys. Of these, one animal had died of an intestinal infection, and the other two, which were healthy adults, were killed by an overdose of Nembutal. In each case the brain was removed immediately after death and fixed in 10 % formol-saline. As degeneration has also been described in human material (Beck, Meyer & Le Beau, 1951), a human brain affected by no apparent pathological condition was fixed in 10 % formol-saline.

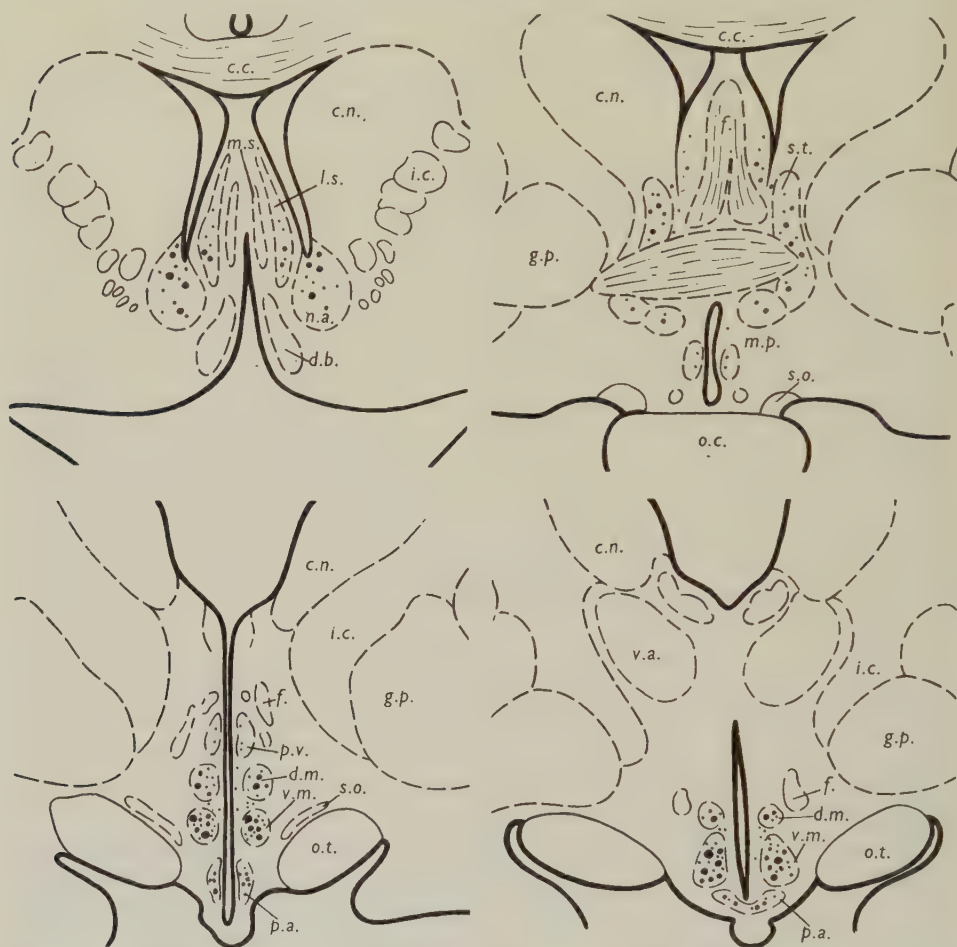
After an adequate period of fixation (2 or 3 weeks) frozen sections cut at 12–20 μ were taken from the level of the septum back to, and including, the mamillary nuclei. These were then prepared by the Glees (1946) ammoniacal silver technique and by the modification of Adey & Meyer (1952). This material was independently examined by the two authors and at each level a careful comparison made with the experimental fronto-hypothalamic material.

No appreciable difference was found between the normal and experimental material in any of the sites examined; as an aid to future work these observations will be described in some detail (Text-fig. 1). In the lateral septal nucleus the 'degeneration' is largely confined to its ventral part and here a few fine fibres, broken up into the characteristic droplets, may be seen in many fields. In the nucleus accumbens and the bed nucleus of the stria terminalis the 'fibre degeneration' is more severe, and in addition an occasional swollen, solid bouton can be seen. Essentially the same appearance is seen in more caudal sections through the medial preoptic and anterior hypothalamic areas, especially near the ventricular margin. The medial septal nucleus and the lateral preoptic area showed no appreciable degeneration. The dorsomedial and ventromedial hypothalamic nuclei, together with the so-called periventricular arcuate nucleus, show extremely marked 'pericellular degeneration'; in addition to numerous 'degenerating fibres' there are many 'swollen, argenthophilic boutons' (Pl. 1, figs. 1, 4). Although an occasional degenerating fibre was seen in the paraventricular nucleus no evidence of 'terminal degeneration' was ever seen in the supraoptic nucleus. The mamillary nuclei, the lateral and posterior hypothalamic areas were likewise free of significant 'degeneration'.

In view of the important implications these findings have on the interpretation of experimental studies on the hypothalamus it should be emphasized that the appearance we have described in normal brains is identical with that found in experimental material. This has been confirmed by other workers who have had experience with silver degeneration techniques, including Sir Wilfrid Le Gros Clark, Dr Paul Glees and Mr D. Simpson.

To clarify our findings in relation to the experimental work on the relevant areas certain points need amplification. First, our material includes only the monkey and the human brain. The finding of terminal degeneration in such nuclei as the bed nucleus of the stria terminalis in lower mammals (e.g. the rabbit and opossum) after olfactory bulb lesions (Le Gros Clark & Meyer, 1947; Adey, 1953) cannot be questioned on these grounds since the authors specifically state that a careful comparison was made with similarly stained normal material. Again, the absence of degeneration in the rostral hypothalamic nuclei in the rabbit after fornix section (Sprague & Meyer, 1950) may be taken as further evidence of a probable species difference. An apparent discrepancy between our findings in the ventromedial nucleus and those of Adey & Meyer (1952) and Meyer (1950) is that these authors state that (a) degeneration is sometimes more severe on the operated than on the unoperated side, and (b) the distribution of the degeneration in the nucleus varied according to the site of the lesion. Regarding the first of these points it has been our experience, both in the normal and the experimental fronto-hypothalamic material, that apart from slight differences in impregnation, the degeneration, as far as can be

judged, is equally severe on the two sides; a quantitative assessment of slight differences in the severity of 'degeneration' is, however, extremely difficult. We have observed differences in the distribution of 'degeneration' within the ventromedial



Text-fig. 1. Diagram showing the distribution of typical 'terminal degeneration' in the preoptic and hypothalamic areas of a normal monkey. The approximate severity of this degeneration is indicated by the size and number of the solid dots.

c.c. corpus callosum
c.n. caudate nucleus
d.b. nucleus of the diagonal band
d.m. dorsomedial hypothalamic nucleus
f. fornix
g.p. globus pallidus
i.c. internal capsule
l.s. lateral septal nucleus
m.p. medial preoptic area
m.s. medial septal nucleus

n.a. nucleus accumbens
o.c. optic chiasma
o.t. optic tract
p.a. periventricular arcuate nucleus
p.v. paraventricular nucleus
s.o. supraoptic nucleus
s.t. bed nucleus of stria terminalis
v.a. nucleus ventralis anterior
v.m. ventromedial nucleus of hypothalamus

nucleus in different sections of the same brain; invariably this has been due to uneven impregnation.

That the technique can still be used to advantage in the hypothalamus is apparent from the following examples. Simpson's (1952) finding of numerous degenerating boutons in the mamillary nuclei after lesions of the fornix system is unequivocal, as a re-examination of his material has shown. Similarly, the description of coarse degenerating fibres in the lateral hypothalamic area by Adey & Meyer (1952) after involvement of the globus pallidus should be accepted as evidence of a pallido-hypothalamic connexion, as we have never observed significant 'degeneration' in either of these two sites. A more serious discrepancy, which cannot at present be accounted for, is the apparent absence of terminal degeneration in any of the hypothalamic nuclei after bilateral ablation of area 10 in the first case reported by Beck *et al.* (1951).

Apart from casting doubt on the validity of the Glees technique so far as it applies to experimental studies on the hypothalamus, the appearance of 'degenerating' axons and terminals in the normal hypothalamus is in itself of interest and merits further study. Among possible causative factors the following might be mentioned, although it is possible, of course, that in a nucleus such as the ventromedial this appearance merely reflects a peculiar staining reaction of functionally normal axons. This appearance may possibly be indicative of the characteristic metabolism of these cell masses; that some hypothalamic nuclei show unusual metabolic activity is well known (as shown, for example, in the appearance of colloid vesicles in the cells of the supraoptic and paraventricular nuclei and the selective staining of the supraoptico-hypophysial tract). In this connexion it might also be recalled that the normal appearance of the cells of the paraventricular and supraoptic nuclei in Nissl-stained material resembles that of chromatolytic neurones (Le Gros Clark, 1938). Another possibility which requires consideration is that the appearance of degeneration is the result of degenerative changes affecting cells during the period of ageing. That such degenerative changes do occur in the central nervous system has been shown by Inukai (1928), who found that the number of Purkinje cells in a rat 1000 days old is about 80 % of that at birth. Against this possibility, however, is the marked severity of the apparent degeneration which we have noted in the dorso-medial and ventromedial hypothalamic nuclei. Finally, it may be that the fibres of these areas are, for some reason, particularly susceptible to the physico-chemical influences which are inherent in the preparation of sections by this histological technique (e.g. formalin fixation and impregnation in ammoniacal silver nitrate).

We wish to thank Prof. Sir Wilfrid Le Gros Clark for his encouragement and advice. The technical assistance of Mr B. Purvis is gratefully acknowledged.

REFERENCES

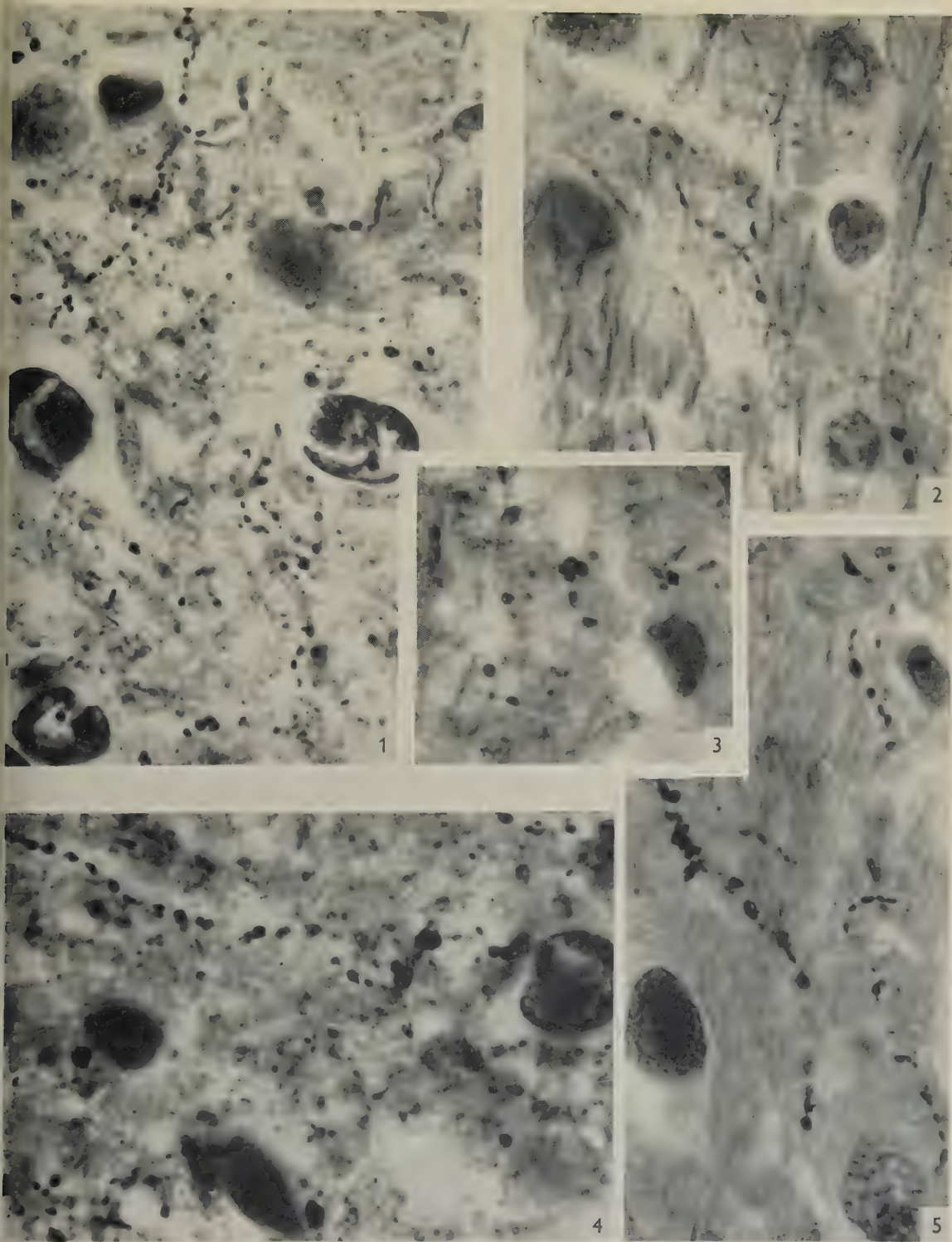
- ADEY, W. R. (1953). An experimental study of the central olfactory connexions in a Marsupial (*Trichosurus Vulpecula*). *Brain*, **76**, 311-336.
ADEY, W. R. & MEYER, M. (1952). Hippocampal and hypothalamic connexions of the temporal lobe in the monkey. *Brain*, **75**, 358-384.
BECK, E., MEYER, A. & LE BEAU, J. (1951). Efferent connexions of the human prefrontal region, with reference to fronto-hypothalamic connexions. *J. Neurol. Psychiat.* **14**, 295-302.

- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new method. *J. Neuropath.* **5**, 54-59.
- INUKAI, T. (1928). On the loss of Purkinje cells with advancing age, from the cerebellar cortex of the albino rat. *J. comp. Neurol.* **45**, 1-32.
- LE GROS CLARK, W. E. (1938). *The Hypothalamus*. Edinburgh: Oliver and Boyd.
- LE GROS CLARK, W. E. & MEYER, M. (1947). The terminal connexions of the olfactory tract in the rabbit. *Brain*, **70**, 304-328.
- MEYER, M. (1950). *Vth International Anatomical Congress, Oxford*, pp. 128-129. Cambridge University Press.
- MEYER, M. & ALLISON, A. C. (1949). Experimental investigation of the connexions of the olfactory tracts in the monkey. *J. Neurol. Psychiat.* **12**, 274-286.
- SIMPSON, D. (1952). The efferent fibres of the hippocampus in the monkey. *J. Neurol. Psychiat.* **15**, 79-92.
- SPRAGUE, J. M. & MEYER, M. (1950). An experimental study of the fornix in the rabbit. *J. Anat., Lond.*, **84**, 354-368.
- WALL, P. D., GLEES, P. & FULTON, J. F. (1951). Corticofugal connexions of posterior orbital surface in Rhesus monkey. *Brain*, **74**, 66-71.

EXPLANATION OF PLATE

All photomicrographs are of normal material stained with the Glees technique.

- Fig. 1. Diffuse 'terminal degeneration' in the ventromedial hypothalamic nucleus of a normal monkey. $\times 1630$.
- Fig. 2. A 'degenerating' fibre in the bed nucleus of the stria terminalis. Normal monkey. $\times 1780$.
- Fig. 3. Swollen, argentophilic boutons in the ventromedial nucleus of a normal human brain. $\times 1780$.
- Fig. 4. A typical 'degenerating' fibre and numerous terminal boutons in the ventromedial nucleus. Monkey. $\times 1950$.
- Fig. 5. Pericellular 'degeneration' in the medial preoptic area. Normal monkey. $\times 1780$.



A STUDY OF SILVER DEGENERATION METHODS IN THE CENTRAL NERVOUS SYSTEM

BY D. H. L. EVANS AND L. H. HAMLYN

Department of Anatomy, University College London

INTRODUCTION

A number of reduced silver methods have been used for the demonstration of degenerating axons and their terminations within the central nervous system. The present study is an attempt to evaluate the relative potentialities of the two methods which the authors have found most useful, namely the Gless and Nauta techniques.

Much of the literature dealing with silver impregnation methods, as applied to the termination of central nervous axons, is concerned with investigations into the structure of the synaptic region. Studies on the appearances of boutons terminaux, both normal and degenerating, have been reviewed by Gibson (1937), and recently by Glees & Nauta (1955). Work by Glees & Le Gros Clark (1941) and Glees (1941, 1942, 1944) has indicated that reduced silver techniques are especially useful for the investigation of neuro-anatomical connexions in experimental material. It is with this aspect that the present paper is concerned.

Following the use of several modifications of the Bielschowsky-Gros technique, Glees developed a reduced silver method that is particularly suitable for the investigation of degenerating material (Glees, 1946). However, this technique also stains normal fibres, with the result that degenerating axons that are scattered through a matrix of normal fibres are not easily seen. This disadvantage has been overcome by a method designed to stain the degenerating axons while suppressing the normal fibre pattern (Nauta & Ryan, 1952), and this technique was later modified to give greater consistency in staining (Nauta & Gyax, 1954).

Although several authors have investigated the degeneration cycle of boutons terminaux in their earlier stages (Hoff, 1932; Foerster, Gagel & Sheehan, 1933; Gibson, 1937), there appears to be little data on the time of persistence of the degeneration material derived from the axonal terminal arborizations or from degenerating axons of passage within the central nervous system. Furthermore, the appearance of degeneration produced by the Nauta technique differs from that given by the Glees method, and in some situations has a different time course.

In the present work the two methods have been used at different sites within the central nervous system, with a view to determining their relative advantages, and to comparing the appearances produced by each. The time course of the degeneration process has been studied with particular reference to the time of persistence of the degeneration material.

It was found that degeneration products stained with the Nauta method persisted for a much longer period than those stained by the Glees method. Additional experiments were therefore carried out in an attempt to investigate the nature of

the degeneration products demonstrated by the two methods. In view of the delayed absorption of the Nauta degeneration material it was thought that it may consist, at least in part, of lipid compounds. The effects of various methods of fat extraction both alone and preceded by fat fixatives were therefore investigated.

For the purpose of this work sites of two sorts have been chosen, some that permit a massive degeneration to be caused, and others where relatively few fibres out of the total become degenerated. Massive degeneration of axonal terminal arborizations occurs in the avian optic tectum following section of the contralateral optic nerve, and in the mammalian gracile and cuneate nuclei after hemisection of the spinal cord. Relatively sparse degeneration is seen in the mammalian cerebral cortex following a lesion of the contralateral hemisphere. In addition to these situations the process of degeneration has been studied in the tractus solitarius and its nucleus following section of the vagus nerve above the nodose ganglion.

MATERIAL AND METHODS

Operative techniques

Avian optic tectum. Adult chickens of various breeds and both sexes were used. For the anaesthetic pentobarbitone sodium (Nembutal) was used as described by Cragg, Evans & Hamlyn (1954), and in addition the orbit was infiltrated with 2 % procaine. The eye was then enucleated using an aseptic technique, and survival times allowed of 3, 5, 9, 11, 13, 15, 19, 28 and 32 days. Following pentobarbitone sodium anaesthesia the brain was removed and the two optic tecta were fixed by immersion in 10 % neutral formol saline. After a fixation time of not less than 7 days frozen sections were cut in the coronal plane at $20\ \mu$ and stored in 10 % neutral formol saline. In view of the complete decussation in the chiasma in birds, the tectum of the ipsilateral side of the lesion was used as the control.

Mammalian material. Adult rabbits of various breeds and both sexes were used. All operative procedures were carried out under pentobarbitone sodium anaesthesia (30 mg./kg. body weight intravenously), supplemented when necessary by open ether. After survival periods ranging from 3 to 170 days the animal was anaesthetized and the whole brain fixed *in situ* by perfusion with 10 % neutral formol saline. The brain was then removed and stored in the same fixative for at least 7 days. All materials were sectioned at $20\ \mu$ thickness with the freezing microtome and the sections stored in the fixative.

Cerebral cortex. The cranium was exposed and trephined over the parietal cortex and an area of 5 mm. diameter of the full thickness of the cortex removed. Survival periods of 5–6 days were allowed. The hemisphere on the side opposite to the lesion was sectioned parasagittally using the procedure described. Control material was obtained from a normal animal at the same time.

The central connexions of the vagus nerve

Degeneration of the sensory fibres of the vagus in the medulla was produced by unilateral excision of the nodose ganglion. This was performed in six rabbits with survival periods ranging from 3 to 170 days.

Terminations of fibres of the gracile and cuneate fasciculi in their respective nuclei

Degeneration in these nuclei was studied in one cat in which the spinal cord had been hemisected at the level of the atlas vertebra 5 days previously.

Staining techniques

Three methods were used: The Bielschowsky-Gros with slight modification, the Glees (1946) and the Nauta & Gyax (1954). As far as the Glees and Bielschowsky methods are concerned, the histological picture produced was the same. The Glees method, however, proved more reliable, and gave a much more even impregnation of the section.

(1) For the Bielschowsky-Gros preparations, the procedure is as follows:

(a) Store the sections in 10 % neutral formol saline and wash in three dishes of distilled water.

(b) Impregnate in 10 % silver nitrate for 15–25 min.

(c) Pass through four baths of 10 % formalin with tap water plus six drops of pyridine per 25 ml. (10 ml. May and Baker 40 % formaldehyde solution and 90 ml. of tap water) until no further white precipitate appears. The time in the formalin baths is critical. The best results are obtained when the sections have become greyish brown in colour. This takes about 15–30 min. The first formalin bath should contain a large volume of fluid (about 200 ml.); trail the sections through this with a glass rod so as to leave most of the precipitate in this bath.

(d) For this stage and until after reduction sections are handled individually. Blot the section and put it into an ammoniacal silver solution for 30–60 sec., made up as follows: 10 % silver nitrate, 5 ml.; 0.880 ammonia drop by drop until no further precipitate appears. Then add two drops excess ammonia.

(e) Wash sections briefly in distilled water.

(f) Reduce in 10 % neutral formol in distilled water.

(g) Wash.

(h) Fix in 5 % sodium thiosulphate for 2 min.

(i) Wash thoroughly.

(j) Dehydrate, clear in creosote and mount in neutral Canada balsam.

(2) The Glees method (1946) was used without modification, except for a quick rinse in distilled water between the ammoniacal silver bath and the reducer.

(3) In general, for the Nauta method, the technique of Nauta & Gyax (1954) was used as described by those authors, except that the preliminary treatment with 15 % ethyl-alcohol was usually left out. This appeared to make no difference to the result.

(4) *Controls.* In the case of the tractus solitarius and nucleus and the gracile and cuneate nuclei, the corresponding normal structures on the opposite side served as a control. For the optic tectum and the rabbit cortex, the controls were separate sections. The experimental sections and the controls were therefore stained at the same time and with the same reagents in two series of dishes until the ammoniacal silver bath was reached. At this stage sections have to be handled individually. The sections were therefore passed through the ammoniacal silver bath and reducer

alternately from the experimental and control series, and then for washing fixation, etc., were again taken into separate dishes.

(5) *Lipid fixation and extraction.* The sites used for this investigation were the tractus solitarius and nucleus and the funiculus cuneatus and nucleus; both Glees and Nauta methods were applied to these. The Glees method was also applied to the avian optic tectum following fat extraction.

For lipid extraction frozen sections were first placed in 50 % alcohol for 12 hr., followed by dehydration in ascending alcohols. The sections were then placed in one of the following mixtures for 90 min.: alcohol-chloroform, 1 : 1; ether-chloroform, 1 : 1; dioxan-iso-propyl alcohol, 1 : 1.

The sections were then brought to water and stained with the methods described.

RESULTS

The general appearances seen with the Glees and Nauta methods

When fully developed the Glees preparations showed a characteristic degeneration appearance against the normal fibre background. The most prominent feature of this was the progressive thickening and fragmentation of the terminal axonal arborizations. Many club and ring-like forms were seen on and near the nerve cell surfaces (Pl. 1, figs. 1, 2). These were absent in the control tectum (Pl. 1, fig. 3). In many instances a fine fibre could be seen in continuity with one of these forms. Whether these endings represent degeneration of the ultimate synaptic structures could not be decided on the evidence available. Massive degeneration of the larger fibre tracts was readily observed in the Glees preparations, but axons in small bundles or in those running individually could not be seen owing to the many normal fibres present.

In critically stained sections the Nauta technique allowed suppression of the staining of the normal fibres (Pl. 1, fig. 5). Consequently, those that were degenerating stood out clearly against a yellow background (Pl. 1, fig. 4). Characteristic of the degeneration was the drop-like disintegration of the fibres described by Nauta & Ryan (1952). Although in the Nauta preparations degeneration was seen in the immediate pericellular region the formations appeared to differ from those of the Glees preparations in that evidence of continuity could be seen. By this is meant that either a stained fragment of the continuing fibre could be observed or its 'ghost-like' outline (Pl. 1, fig. 6).

Time course of degeneration in different sites

Avian optic tectum. In this situation both Glees and Nauta degenerations were delayed in appearance compared with the other sites studied. The Glees preparations showed only increased affinity for silver staining of the interrupted optic axons after 5 days, and the fully established picture of degenerating terminal arborizations was observed only after a 9 to 11-day interval. From 15 days onwards the degenerating material was decreased in quantity, and by 28 days only a little granular debris and some fragments of thicker fibres were visible.

Sections taken from the same block were also stained by the Nauta method. After

5 days' degeneration this technique showed only a few fibres, not yet in process of disintegration in the optic fibre layer only (Pl. 2, fig. 7). As the control sections were completely negative (Pl. 4, fig. 20) staining of these fibres was presumably due to the increased argyrophilia already noted in the early Glees preparations. At 9 days evidence of fragmentation and drop-like disintegration was apparent and by 28 days (Pl. 2, fig. 8) full Nauta degeneration was established and reached the same depth from the tectal surface as that seen in the Glees preparation. Control sections were at all times completely negative and there was no staining of normal fibres.

Mammalian preparations

The Nauta staining was consistent in the sites studied. Degeneration was seen as early as 3 days, following the lesion and was fully established by 5 days (Pl. 1, fig. 4). In the case of the Glees preparations positive results were obtained only in the cuneate and lateral cuneate nuclei.

In the cerebral cortex, the dense felt-work of the normal neuropil masked any sign of degeneration with the Glees method and even with the Nauta slides as a guide, no evidence of degeneration could be found (Pl. 3, fig. 18).

Repeated attempts were made to obtain evidence of degeneration in the tractus solitarius and nucleus with the Glees method. Although the site could be exactly localized by means of Nauta preparations from the same block, no appearances of degeneration were observed in the Glees material at the times examined (3, 4, 5, 17 and 45 days).

Time of persistence of Nauta degeneration material

The process of disappearance of degeneration products has been studied in the tractus solitarius and its nucleus following supranodose (extracranial) vagotomy. Survival periods ranged from 3 to 170 days. The quantity of degeneration material, which was maximal at 5 days, showed little change up to 45 days (Pl. 2, fig. 9). It was diminished at 112 days (Pl. 2, fig. 10), and by 170 days it had completely disappeared (Pl. 4, fig. 21). It was interesting to note that the degeneration was most persistent in the nucleus, whereas it was absent in the tract at an earlier stage (112 days).

The effect of fat extraction on the staining techniques

In view of the observed differences in the morphology and time course of the two types of degeneration material, it was thought that the chemical processes involved might be different. The long persistence of the degeneration in the case of the Nauta method suggested that lipid elements were being stained.

To investigate this possibility, treatment with fat solvents was carried out as already described, the sites concerned being the avian optic tectum and cuneate nuclear complex. The results were very different in the Nauta preparations compared with the Glees. Of the fat solvents used, the alcohol-chloroform mixture was found to be the most effective. In the case of the Glees preparations, preliminary treatment with this mixture resulted in an overall improvement in staining compared with the non-treated controls (Pl. 4, figs. 23, 24). The background was clearer,

and the neuropil and fibre tracts more uniformly stained. The characteristic degeneration forms were easily observed and undiminished in density.

The effect of using the same solvent prior to carrying out the Nauta process was to abolish completely the staining of degenerated fibres (Pl. 2, fig. 11), whereas these were seen in abundance in the non-treated controls (Pl. 2, fig. 12).

The ether-chloroform and dioxan-isopropyl alcohol mixtures produced similar results but were less effective, the Nauta staining being only incompletely abolished.

These results indicate that the Nauta technique stains altered lipid substances in the degenerating nerve fibres. At the same time the persistence of the Glees degeneration products following fat extraction shows that the chemical processes are different and do not involve the staining of lipid substances.

An attempt to fix the lipids before fat extraction and Nauta staining was made by preliminary mordanting of the sections in 5% potassium bichromate for 2 days. This enabled the Nauta degeneration material to be stained in spite of the fat extraction (Pl. 3, fig. 13). But in contrast to the homogeneous yellow background normally found, additional elements were stained in non-degenerated regions. In tracts where large fibres were seen in transverse section (medial lemniscus, normal tractus cuneatus), examination under low power showed black rings surrounding the nerve fibres. At higher magnifications the rings were seen to be crenated and only incompletely enclosed the light brown axon. On examination in optical section, it was seen that the distance between the inner margin of the ring and the axon varied, suggesting that the ring was part of a reticulated structure (Pl. 3, fig. 14). This was confirmed by examining fibres in the nucleus cuneatus which were running in the plane of the section. A coarse and wide-meshed reticulum was seen surrounding the axon, apparently occupying the region of the myelin sheath (Pl. 3, fig. 15; Pl. 4, fig. 22), although the latter was not stained.

The appearances of the degenerating tractus cuneatus were different in that as well as the reticulum which was found in the normal regions of the section, the axonal degeneration characteristic of the Nauta stain was also present.

Closer examination showed that the reticulum in this degenerating tract was in the process of disintegration. In transverse section it could frequently be seen that the reticular ring surrounded a darkly staining axon, itself in process of degeneration (Pl. 3, fig. 16).

DISCUSSION

Time course of the degeneration process

The time of appearance of degeneration of axons and their terminal arborizations as studied by reduced silver methods varies to some extent between different species, and more particularly between different classes of animals. In mammals Glees & Nauta (1955) point out that corticofugal fibres will degenerate to the stage of 'drop-like' disintegration within 5 days in the rat and cat, but in the monkey require upwards of 7 days to reach the same stage. Armstrong (1950), in a study of the visual pathways in reptile, noted that after eye enucleation no changes were observed in the axons of the optic tract earlier than 7 days following operation, and that this was followed by a period of 6 days, during which the only change was a progressive increase in the affinity of the axons for silver. Evidence, in the form of

fine rings, of the degeneration of the terminal aborizations within the lateral geniculate nucleus was first seen on the sixth day, and from this time onwards thickening and fragmentation of the aborizations was progressive. The degeneration material had mainly disappeared by 25 days and completely at 10 weeks. The time, course and appearances of this degeneration cycle correspond closely to that described in the present paper for the avian optic tectum, in preparations made with the Glees method. A further point of similarity is that both in the reptile and bird it was not possible to stain the ring- or club-like forms in normal material, and in the case of the avian tectum, the terminal aborizations themselves failed to appear in the normal specimens. However, in this connexion, it is of interest that signs of degeneration of terminal boutons have been described in the stellate ganglion of cephalopods as early as 13 hr. after interruptions of the nerve fibres (Sereni & Young, 1932). As these animals are poikilothermous such unexpectedly early changes may be attributable to the relatively high temperature of the sea water of their environment (around 25° C.).

When the Nauta method was used on avian material the appearances of degeneration were not fully established until about 28 days following the operation. This delay was not experienced in any of the mammalian experiments, and it has not been possible to trace any reference to it in the literature. Such a considerable variation in the time of appearance of the degeneration material in the two methods suggests that different chemical processes are involved. This possibility is reinforced by the long time of persistence of the Nauta degeneration. In the nucleus of the tractus solitarius following supra-nodose vagal section, much fragmented axonal material was still to be seen even at 112 days after the operation.

Appearances and nature of the degeneration material stained

Preliminary treatment of the sections with fat solvents emphasizes that there is a difference in the chemical processes involved in the two staining procedures. It was first thought that this finding could be best explained by assuming that the Nauta method was staining only the degenerating myelin sheath. Examination of sites containing large degenerating fibres was consistent with this view, but where finer fibres are also present (e.g. Pl. 3, fig. 17) there can be no doubt that the degenerating axons are also involved in the staining process. Similar evidence is seen in sites where finer axons are to be expected, such as in pericellular aborizations (Pl. 2, fig. 12). It seems then that the Nauta method stains both degenerating axons and myelin.

Even when fully established, the Nauta degeneration picture appeared different from that of the Glees. 'Drop-like' disintegration of the axons with fragmentation and vacuole formation were characteristic (Pl. 1, fig. 6) of the former method. The club- and ring-like forms and finer ramifications of the aborization, which were a prominent feature in the Glees preparations of 5-15 days (Pl. 1, fig. 2), were at no stage to be seen in Nauta material. In fact, the impression was formed that the Nauta method showed the disintegration of the main axons and their branches, but did not stain their finest ramifications.

This could be due to two factors. One of these is that if the myelin sheath is not present on the final aborizations no contribution to the degeneration material can

be expected from this source. However, recent work using the electron microscope indicates that even the finest so-called non-medullated fibres possess a sheath which may represent a single myelin lamella (Gasser, 1952; Fernandez-Moran, 1952). It may be that the absolute amount of lipid substances in the finest fibres is so small that it appears as scattered granules and therefore only gives an amorphous appearance.

The involvement of lipid materials in the Nauta staining process also invites comparison with the Marchi technique. For tracing degenerating myelinated tracts, the latter has the advantage that serial sections can be used. However, in situations where the degenerating fibres are diffused, interpretive difficulties are experienced, particularly in view of the relative ease with which artefacts are produced with the Marchi method. The Nauta method is more reliable and gives as a result of its axonal component a clearer picture of the degenerating pathway. In addition, this technique demonstrates the degenerating fibres as far as their pericellular ramifications and because the nerve cells are also stained, interpretation is easier.

*The applications of the Glees and Nauta methods to investigation
of neuro-anatomical pathways*

With regard to the relative usefulness of the two methods in tracing neural pathways, it was found that the Glees method was very suitable, when successful, for demonstrating the exact site of the terminal arborization. The expression 'terminal degeneration' has been avoided, as it was not always certain that the ring- and club-like forms seen were terminal structures in the synaptic sense. Armstrong (1950), in his study of the reptile visual pathways, also experienced this difficulty. The main limitation of the Glees technique was in tracing axons of passage. This was due to the staining of the normal neuropil, which frequently masked the appearances of degeneration, as may be seen in the case of the cerebral cortex (Pl. 3, fig. 18), which should be compared with the Nauta preparation from the same block (Pl. 3, fig. 19).

It is in this respect that the Nauta method is complementary to the Glees. The complete suppression of the staining of normal fibres permits easier recognition of the criteria of degeneration as described by Glees & Nauta (1955). In addition, the suppression of the normal fibres allows the use of much thicker sections (up to 30μ in some situations) than would otherwise be possible. This is a considerable advantage in tracing the course of fibres over long distances. However, it must be emphasized that when using either the Glees or the Nauta methods the careful use of control material is essential.

SUMMARY

1. A comparison has been made between the Glees and Nauta silver degeneration methods in the central nervous system. This has been carried out in relation to the morphological appearances produced, the time course of the degeneration cycle and the effect of lipid solvents on the staining.

2. In Glees preparations the characteristic appearances showing club- and ring-like forms, as described by previous authors, were confirmed. The Nauta method

demonstrated clearly, with complete suppression of the normal fibre background, the course of degenerating axons and their branches. However, in our experience the finest degenerating ramifications, shown by the Glees technique, were not stained in Nauta preparations. This interpretation is partly based on the apparent terminal appearance of the Glees degeneration forms compared with the probable derivation from axonal and myelin fragments as seen in the Nauta.

3. The time course of the degeneration cycle has been studied in the avian optic tectum and in the tractus solitarius and its nucleus in the rabbit. In the former the time of establishment of the Glees degeneration appearances was delayed to 7–11 days following operation, compared with 3 days for mammalian material. In the tectum the Glees degeneration products had virtually disappeared by 28 days. In the same site the Nauta degeneration picture became fully established only by 28 days. With regard to the time of persistence of Nauta degeneration material in the tractus solitarius and nucleus, this was still present at 112 days but had disappeared by 164 days.

4. The effect of preliminary treatment of the frozen sections with fat solvents was to abolish the Nauta staining, whereas that of Glees preparations was unimpaired or even improved. Taking into account the variation in morphological appearance and time course in the two methods, combined with the effect of fat solvents, it is considered that the underlying chemical processes involved in the two methods must be different.

5. It is concluded that the Glees method is satisfactory for studying the exact site of termination of axonal arborizations in appropriate regions. It is at a disadvantage in situations where scanty degeneration is present amongst a mass of normal fibres. The Nauta method is most useful where fewer degenerating fibres must be traced through a matrix of normal fibres as the staining of the latter is suppressed, but it fails to demonstrate degeneration in the finest branches.

We wish to thank Professor J. Z. Young and Mr K. C. Richardson for valuable advice and criticism and Mr J. A. Armstrong and Miss Thelma Marchment for help with the photomicrography.

REFERENCES

- ARMSTRONG, J. A. (1950). An experimental study of the visual pathways in a reptile (*Lacerta vivipara*). *J. Anat., Lond.*, **84**, 146–167.
- CRAGG, B. G., EVANS, D. H. L. & HAMLYN, L. H. (1954). The optic tectum of *Gallus domesticus*: A correlation of the electrical responses with the histological structure. *J. Anat., Lond.*, **88**, 292–306.
- FERNANDEZ-MORAN, H. (1952). The submicroscopic organization of vertebrate nerve fibres. *Exp. cell Res.* **3**, 282–359.
- FOERSTER, O., GAGEL, O. & SHEEHAN, D. (1933). Veränderungen an den Endösen im Rückenmark des Affen nach Hinterwurzel durchschneidung. *Z. ges. Anat. u. Z. Anat. Entw. Gesch.* **101**, 553–565.
- GASSER, H. S. (1952). *Cold Spr. Harb. Symp. quant. Biol.* **17**, 32–36.
- GIBSON, W. C. (1937). Degeneration of the boutons terminaux in the Spinal Cord. *Arch. Neurol. Psychiat.*, *Chicago*, **38**, 1145–1157.
- GLEES, P. (1941). Termination of optic fibres in the lateral geniculate body of the cat. *J. Anat., Lond.*, **75**, 434–440.
- GLEES, P. (1942). The termination of optic fibres in the lateral geniculate body of the rabbit. *J. Anat., Lond.*, **76**, 313–318.

- GLEES, P. (1944). The anatomical basis of cortico-striate connections. *J. Anat., Lond.*, **78**, 47-51.
- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath.* **5**, 54-59.
- GLEES, P. & LE GROS CLARK, W. E. (1941). Termination of optic fibres in the lateral geniculate body of the monkey. *J. Anat., Lond.*, **75**, 295-308.
- GLEES, P. & NAUTA, W. J. H. (1955). A critical review of studies on axonal and terminal degeneration. *Msschr. Psychiat. Neurol.* **129**, 74-91.
- HOFF, E. C. (1932). Degeneration of the boutons terminaux in the spinal cord. *J. Physiol.* **74**, 4-5 P.
- NAUTA, W. J. H. & GYGAX, P. A. (1954). Silver impregnation of degenerating axons in the central nervous system: a modified technique. *Stain. Tech.* **29**, 91-93.
- NAUTA, W. J. H. & RYAN, L. F. (1952). Selective silver impregnation of degenerating axons in the central nervous system. *Stain. Tech.* **27**, 175-179.
- SERENI, ENRICO & YOUNG, J. Z. (1932). Nervous degeneration and regeneration in cephalopods. *Pubbl. Staz. zool. Napoli*, **12**, 1-36.

EXPLANATION OF PLATES

PLATE 1

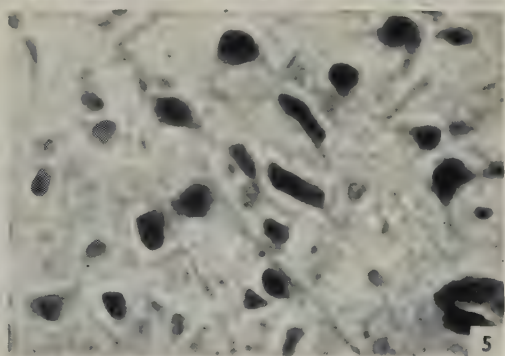
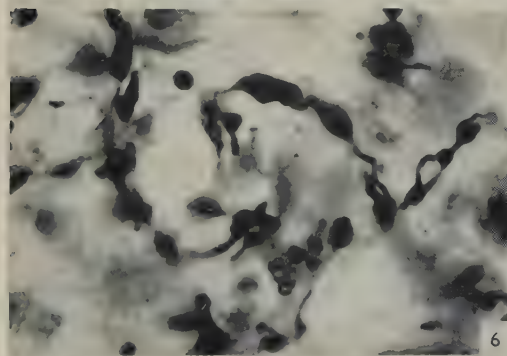
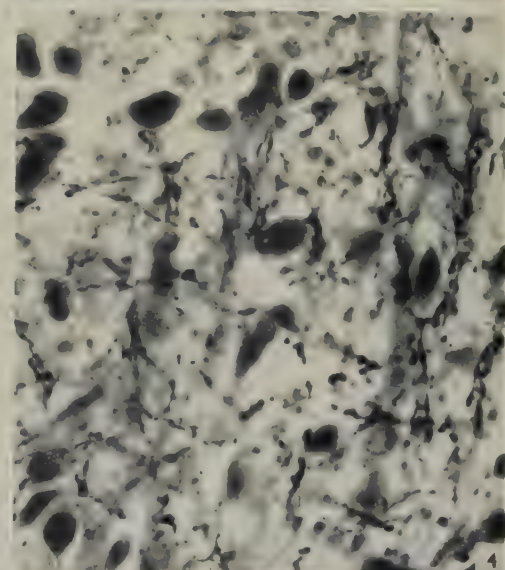
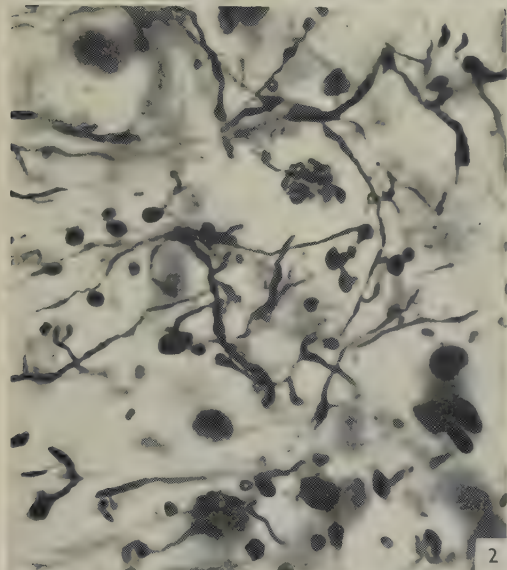
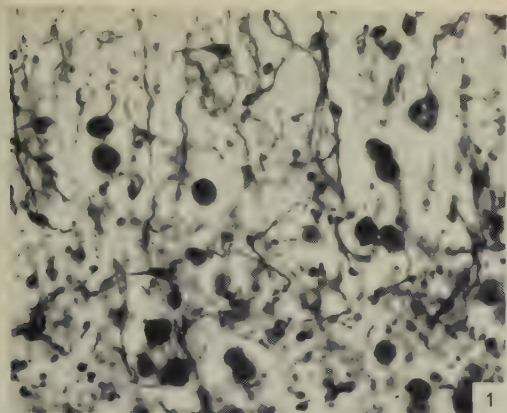
- Fig. 1. Avian optic tectum, 11 days following contralateral optic nerve section. Appearances of degeneration of the terminal arborizations of the optic nerve afferents are seen throughout the field. Glees method. $\times 552$.
- Fig. 2. Higher power of the same preparation as fig. 1. Club- and ring-like forms of various sizes are seen. $\times 1320$.
- Fig. 3. Control of figs. 1 and 2. This is the contralateral optic tectum in which the clubs and rings are absent. Glees method. $\times 552$.
- Fig. 4. Nucleus of the tractus solitarius, 5 days after supra-nodose section of the vagus nerve. Numerous nerve fibres are in process of degeneration. Nauta method. $\times 552$.
- Fig. 5. Control of fig. 4. The contralateral nucleus of the tractus solitarius in the same section. In this normal field no nerve fibres are stained. Nauta method. $\times 552$.
- Fig. 6. High power of the lateral cuneate nucleus 5 days after ipsilateral hemisection of the spinal cord at the level of C. 1. The droplets and vacuoles formed along the course of the degenerating axons are seen. Nauta method. $\times 1320$.

PLATE 2

- Fig. 7. Avian optic tectum, 5 days' degeneration. At this stage the staining of nerve fibres does not extend deep to the optic fibre layer (*o.f.l.*). Nauta method. $\times 552$.
- Fig. 8. Avian optic tectum, 28 days' degeneration. The optic fibre layer is now packed with degenerating nerve fibres which extend into the deeper layer. $\times 552$.
- Fig. 9. Nucleus of the tractus solitarius, 45 days after supra-nodose vagotomy. There is slight diminution of the degeneration material compared with fig. 4. $\times 552$.
- Fig. 10. Nucleus of the tractus solitarius 112 days after supra-nodose vagotomy. Considerable diminution in the quantity of the degeneration material is now apparent. Nauta method. $\times 552$.
- Fig. 11. Cuneate nucleus 5 days after ipsilateral hemisection of the spinal cord at the level of C. 1. Following treatment of the sections with fat solvents no degenerating fibres are seen. Nauta method. $\times 552$.
- Fig. 12. Control of fig. 11. Many degenerating fibres are seen in this field. Nauta method without fat extraction. $\times 552$.

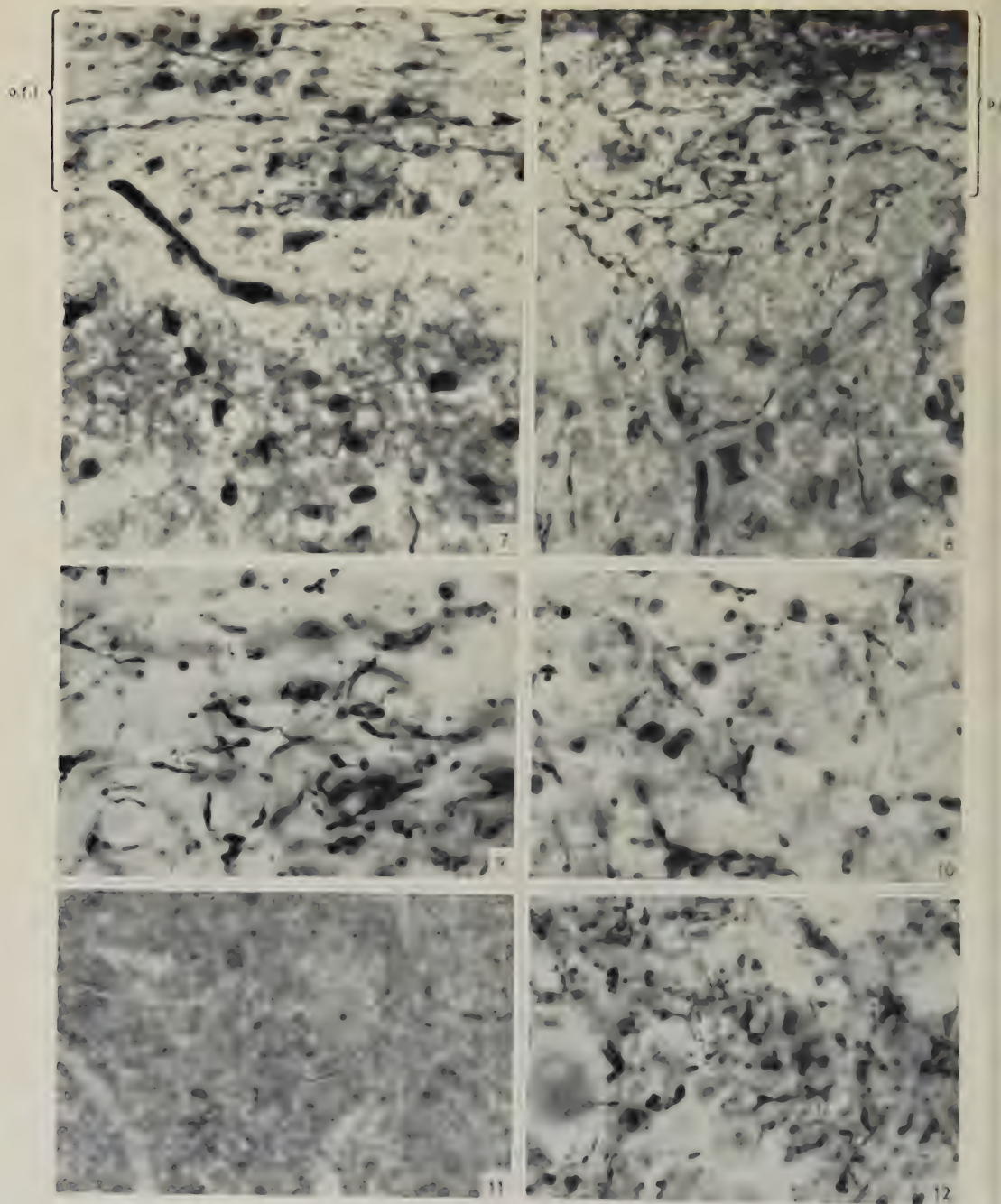
PLATE 3

- Fig. 13. Cuneate tract, 5 days' degeneration. Preliminary treatment with potassium bichromate has preserved the staining of the degeneration material in spite of the use of fat solvents. Nauta method. $\times 552$.
- Fig. 14. Normal cuneate tract after the same treatment as fig. 13. An incomplete ring of darkly stained material is seen surrounding the faintly stained axons. $\times 1080$.
- Fig. 15. Material from the same specimen as fig. 14 but sectioned longitudinally. The dark staining material is seen to form a reticulum surrounding the axon. Nauta method preceded by mordanting and fat solvents. $\times 1080$.

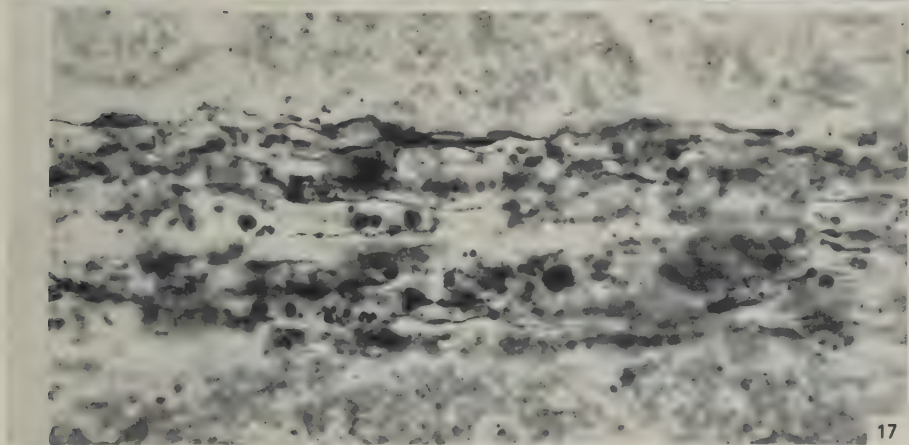
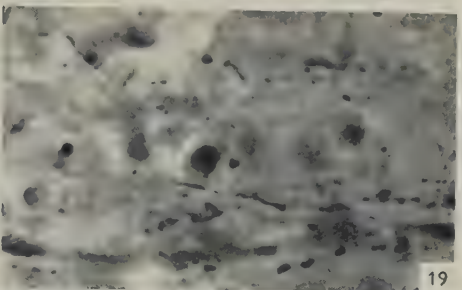
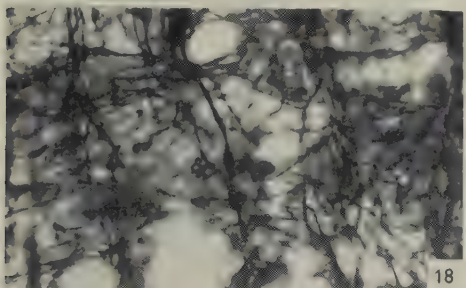
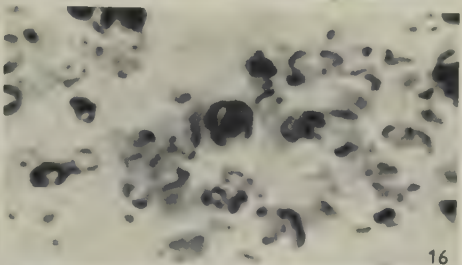
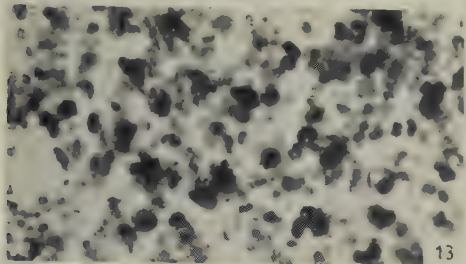
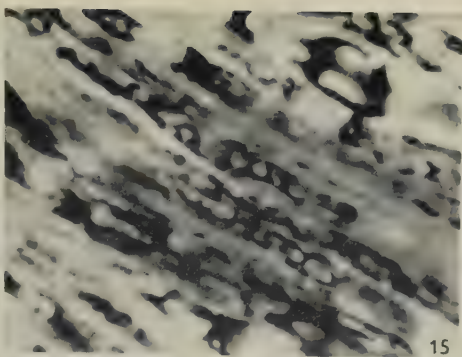
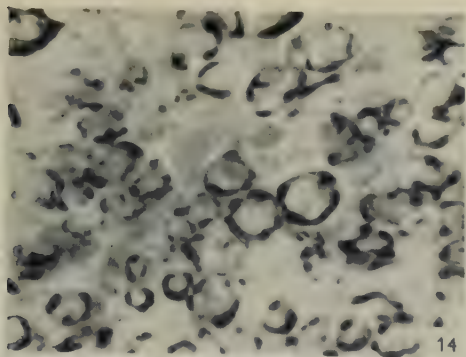


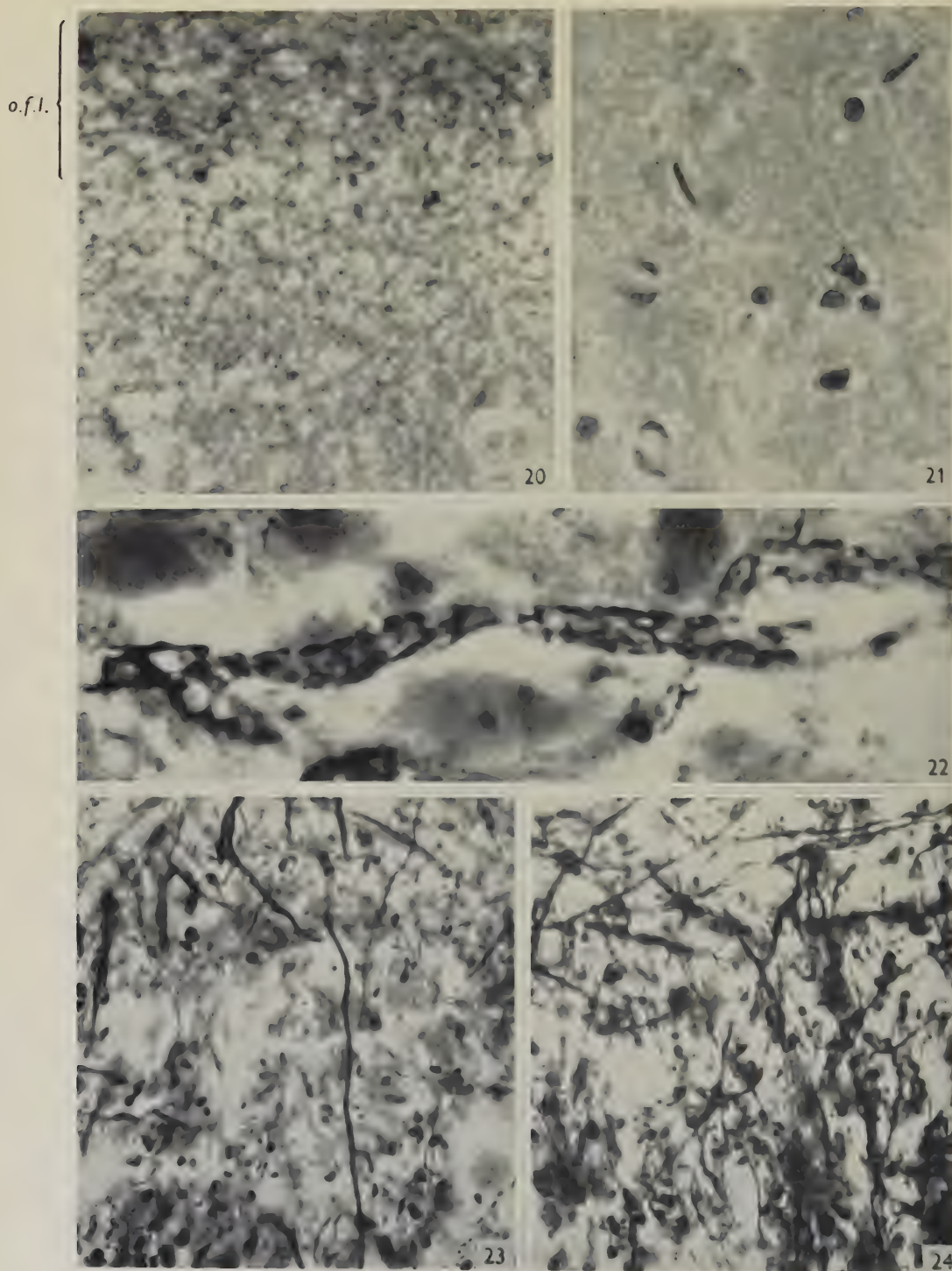
EVANS AND HAMLYN—SILVER DEGENERATION METHODS IN THE NERVOUS SYSTEM

(Facing p. 202)



EVANS AND HAMLYN—SILVER DEGENERATION METHODS IN THE NERVOUS SYSTEM





- Fig. 16. Cuneate tract, 5 days' degeneration. The degenerating axon in the centre of the field is seen to be surrounded by the reticulum. Nauta method preceded by mordanting and fat solvents. $\times 1080$.
- Fig. 17. Transverse section of medulla oblongata, 5 days after supra-nodose vagotomy, showing a degenerating vagal rootlet cut in longitudinal section. The staining of the normal fibres of the surrounding medulla is suppressed. Nauta method. $\times 552$.
- Fig. 18. Rabbit cerebral cortex, 5 days after a lesion of the contralateral hemisphere. No evidence of degeneration can be seen in the densely stained neuropil. Glees method. $\times 552$.
- Fig. 19. A section from the same block as fig. 18 showing some degenerating fibres. The staining of the normal neuropil is suppressed. Nauta method. $\times 552$.

PLATE 4

- Fig. 20. Avian optic tectum (control of fig. 7). No fibres are stained in the optic fibre layer (*o.f.l.*). Nauta method. $\times 690$.
- Fig. 21. Nucleus of the tractus solitarius, 170 days' degeneration. There is now no staining of degenerating material. Nauta method. $\times 690$.
- Fig. 22. A further view of the reticulum surrounding a longitudinally disposed normal axon in the nucleus cuneatus. Nauta method preceded by mordanting and fat extraction. $\times 1650$.
- Fig. 23. Cuneate nucleus, 5 days' degeneration. Numerous degeneration forms typical of the Glees method are seen. Glees method preceded by fat extraction. $\times 690$.
- Fig. 24. Section from the same block as fig. 23, stained by the Glees method without preliminary fat extraction. $\times 690$.

THE IMMEDIATE EFFECTS OF LIGATURE OF VASA NERVORUM

By MICHAEL J. BLUNT* AND KATHLEEN STRATTON

*Departments of Anatomy and Physics,
Royal Free Hospital School of Medicine*

Several attempts have been made to assess the relative importance of the vasa nervorum and the intrinsic longitudinal vascular plexuses of nerve in maintaining the blood supply of a segment of nerve trunk. Okada (1905) and Koch (1926) considered that the regional supply was the more important of the two. Okada based his conclusions on the fact that ligature of regional vessels produced degeneration of nerve fibres, and Koch on his interpretation of the effects of vascular occlusion on the current of injury in nerve. On the other hand, Adams (1943) obtained mainly contrary results from experiments similar to Okada's and stated that he could find no degeneration or, 'at the most, degeneration of relatively few solitary fibres'. Bacsich & Wyburn (1945*a, b*) also concluded that the regional vessels were of only secondary importance. Their criteria were the appearance of the intraneural plexuses and the rate of regeneration of axons in segments of nerve deprived of their regional blood supply.

The evidence to date has necessarily been of an indirect nature, and it was thought that a more direct guide to the relative importance of different vascular factors might be obtained by using the clearance of ^{24}Na as an index of circulatory efficiency. The results of a series of acute experiments using such a method are reported in this paper.

MATERIAL AND METHODS

Rabbits of mixed breeds, of both sexes, and of weights ranging from 1200 to 2500 g. were anaesthetized with intravenous urethane, and the sciatic nerve was exposed in the thigh and gluteal region through a standard lateral approach. The nerve was carefully lifted from the underlying muscles, without damage to its blood vessels, and several layers of thin waxed paper were placed under it, to insulate it from contact with surrounding tissues. The tibial component of the sciatic nerve trunk was then injected, halfway down the back of the thigh, with Krebs-Ringer solution (Umbreit, Burris & Stauffer, 1949) containing ^{24}Na prepared by irradiation of solid NaCl at A.E.R.E., Harwell. Injections were made from an Agla micrometer syringe, and their volume varied from 0.001 to 0.01 ml. The radioactivity of the solution varied from about 800 $\mu\text{c./ml.}$ to about 80 $\mu\text{c./ml.}$ During the injection the waxed paper was protected by a layer of filter-paper and this, together with the top layer of waxed paper, was removed on completion of the injection. Any backflow along the needle track was removed by dabbing with filter-paper.

* Present address: The Department of Anatomy, Medical College of St Bartholomew's Hospital.

A Perspex platform 0.5 cm. thick, with a central aperture 1 cm. in diameter, was set up as close as possible over the operation field, by means of adjustable side supports. The distance of the platform from the injection site ranged from about 1 cm. to a maximum of 2 cm. An end-window G.M.4 Geiger counter was placed over the aperture and closely shielded with lead bricks. An A.E.R.E. Type 1221 B Dekatron scaling unit was used for recording the counts in most of the experiments, and an A.E.R.E. Type 200 A scaling unit for the remainder.

The radioactivity over the injection site was recorded by means of β counts of 30 sec. duration repeated at 1 min. intervals. Counts were recorded during two consecutive periods of approximately 20 min., commencing 7 min. after the injection. At the end of the first counting period one of various experimental procedures was carried out, and a second layer of waxed paper was removed and kept for checking the background radioactivity at the end of the experiment. After an interval, usually of from 1 to 5 min., counting was resumed, and at the end of the second counting period the sciatic nerve was removed. Estimations of the background radioactivity for both periods of the experiment were then obtained by taking the mean of three half-minute counts over the operation field, first with the final layer of waxed paper in place, and then with this replaced by the layer removed at the end of the first period of the experiment.

The observed counting rates were corrected for the paralysis time of the scaling unit used, and the appropriate background counting rate was subtracted. The corrected counting rates were plotted semi-logarithmically against time as in Fig. 2. The resulting curves were found to be approximately linear for each of the two separate periods of an experiment, and the slope of the line for each experimental period was calculated by the method of least squares. This slope will be called the clearance slope (K_1 or K_2). Occasional counts were missed (Fig. 2, K_2) apart from those deliberately omitted in the first 6 min. after injection, but the total numbers of observations used in the calculation of each parameter are shown in the tables. An increase or decrease in the general level of counting rate occurring immediately after operative interference with the blood supply to the nerve has sometimes been observed (Fig. 2). This was due to an unavoidable minor alteration in the distance of the nerve from the counter tube occasioned by the operative procedure, but during each counting period the position of the nerve relative to the counter remained constant. Such variations in distance were always small (perhaps 1–2 mm.) compared with the total distance of the nerve from the counter tube, and although they affected the counting rate they did not affect the clearance slope values obtained.

Since the initial clearance slopes vary considerably, the possible differences between these slopes and the final slopes must be studied by a statistical method that makes allowances for the variation in the initial slopes. Covariance analysis (Snedecor, 1946) provides a suitable method and has been used throughout this work for testing the differences between clearance slopes. In order to present the results more clearly, however, the mean values of K_1 and $(K_1 - K_2)$ were calculated for each group of experiments, and the change in clearance slope was expressed as a percentage of the value of the mean initial clearance slope.

Records of rectal temperature were maintained throughout each experiment, room temperature was kept constant, and the operative field was shielded from

draughts. Any experiment in which the rectal temperature varied by more than 1° C. was discarded from the series.

Experiments were designed to provide information on the form of the blood supply to the rabbit sciatic nerve, possible routes of clearance of ^{24}Na from the injection site, and the effects on the clearance of ^{24}Na of various kinds of operative interference with the blood supply of the nerve.

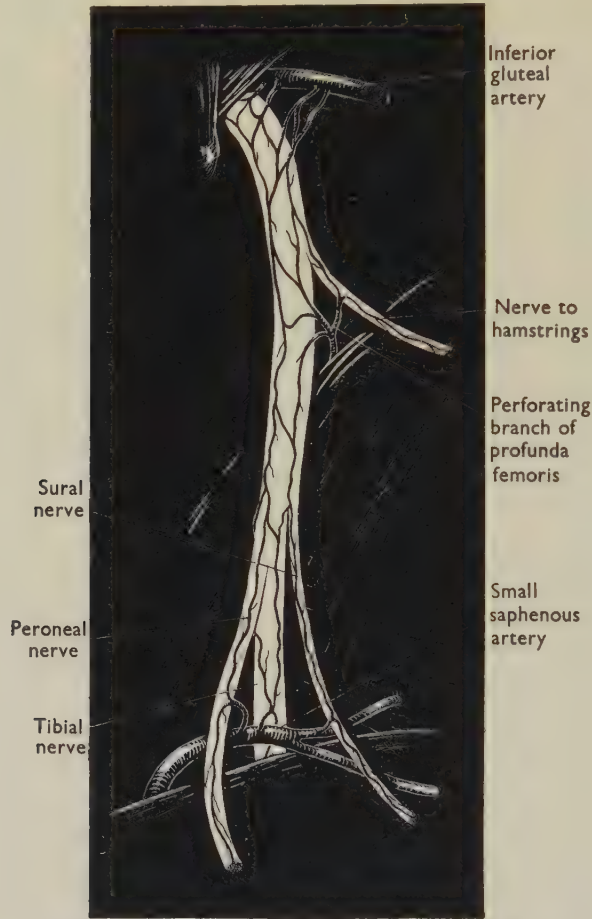


Fig. 1. The arterial blood supply to the rabbit sciatic nerve trunk in the thigh.

RESULTS

A. The pattern of the blood supply to the rabbit sciatic nerve

In the pelvis, the sciatic nerve and its roots were supplied by branches of the hypogastric artery. In the thigh, the findings of Adams (1943) were confirmed and amplified by the material of this investigation (Fig. 1). One or two vessels from the inferior gluteal artery reached the nerve in the gluteal region, and the lowest of these linked up with a perforating branch of the profunda femoris in the upper part of the thigh. At the lower end of the thigh the nerve was supplied by branches of the small

saphenous artery, and usually these branches reached the main trunk by travelling with each of its terminal divisions and with the sural nerve. In nine out of sixty-one sciatic nerves examined the tibial nerve received a short second branch from the popliteal artery. The arteries were all closely accompanied by veins, and adjacent vessels of supply were linked by longitudinal anastomoses in the epineurium. These longitudinal vessels, of which there were usually two, were joined to one another by transverse branches, and they gave off interfascicular vessels to both parts of the sciatic nerve trunk.

In the leg, only small vessels supplied the terminal divisions. The peroneal nerve received a small branch of the anterior tibial artery, and the tibial nerve received no further vessels until just above the medial malleolus, where it was supplied by a branch of the great saphenous artery.

B. Possible routes of clearance of ^{24}Na from the injection site

It was considered that ^{24}Na might be cleared from the injection site by diffusion along the length of the nerve trunk, and possibly by removal into the cerebro-spinal fluid, by direct diffusion into surrounding tissues, and by clearance along vascular pathways. Experiments were carried out to investigate the possibility of clearance by each of these routes.

At the end of three experiments the nerve was cut into 1 cm. segments and the β activity of each segment was estimated. In all three cases it was shown that, during the period of the experiments, the depot of radioactive sodium remained localized in a length of nerve trunk less than 1 cm. on either side of the injection site. Therefore, during the period of the experiments the bulk of radioactive material did not diffuse beyond the effective β counting range of the Geiger counter.

In two rabbits lumbar puncture was performed, and five rabbits were subjected to cisternal puncture at the end of experiments. In all of these animals the radioactivity of the cerebro-spinal fluid was less than that of an equivalent volume of blood. Taking this evidence in conjunction with the findings on localization of the injected solution it was concluded that there was no evidence of direct clearance to the cerebro-spinal fluid.

The waxed paper placed between the nerve trunk and the underlying muscles was shown to provide effective insulation from direct clearance into the surrounding tissue fluid, for in two animals the nerve trunk was tied both at the upper and lower ends of the thigh after injection, and it was found that clearance was effectively stopped.

The first three possibilities were thus excluded as factors likely to effect clearance to a significant extent, and the only remaining possibility was that of clearance by way of vascular pathways.

C. Effect of volume of injection

Initial clearance slopes in all experiments were found to be unrelated to the volume of injection used, within the limits employed. During the first 6 min. after the injection it was sometimes found that clearance was particularly rapid, and it was considered that this may have been due to a slightly increased pressure gradient in the tissue fluid during the first few minutes after the injection: the first 6 min. after

the injection were therefore always ignored in the calculation of the clearance slopes. There was, however, no relation between an initially steeper clearance slope and the volumes of injection employed. When the larger volumes were injected it was found that more of the fluid leaked back along the needle track and this was removed immediately by dabbing with filter-paper.

D. Background radioactivity

In only five out of fifty-one experiments was the background more than 20% of the final counting rate; in twenty-eight experiments it was less than 10%. The proportion of the background count due to contamination of the waxed paper was less than a third of the total background count in each of five experiments where this was tested.

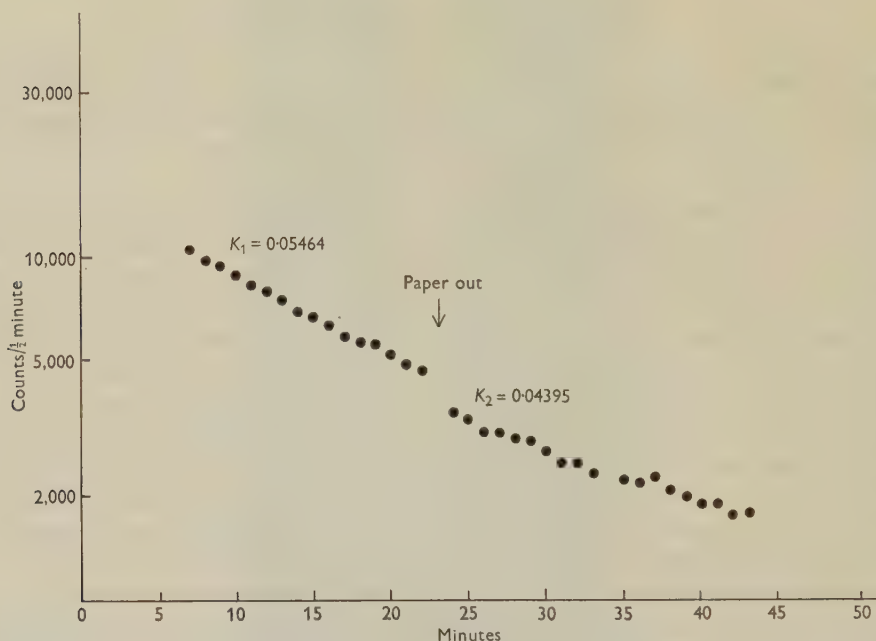


Fig. 2. Group 1, control, Exp. 6.

E. The ^{24}Na clearance estimations after interference with vascular supply Group 1. Control (Fig. 2, Table 1)

In seven preliminary experiments counting was pursued uninterruptedly for periods of from 45 to 70 min. after injection of the radioactive Krebs-Ringer solution. In four experiments it was found that, over this range of time, there was a gradual deviation from linearity in the semi-logarithmic curve, the graph showing a gentle convexity towards the origin. Over a 20 min. period, however, there was a good approximation to linearity. For this reason it was arbitrarily decided to separate all the experiments into two periods and to carry out any experimental procedures at the end of the first period.

In the control series, in which no kind of vascular interruption was carried out, an alteration in the mean initial clearance slope for the group of 16.81% was revealed by this method of treatment of the results.

Group 2. Ligature of the nerve trunk inferiorly (Fig. 3, Table 1)

At the end of the first period of the experiments the divisions of the sciatic nerve trunk and the sural nerve were ligated together above the entry of the vasa nervorum from the small saphenous artery: all the blood supply from below was thus occluded, leaving the nerve supplied only by intrinsic longitudinal vessels from above. A clearance slope alteration of 53.32% was found for this group of experiments.

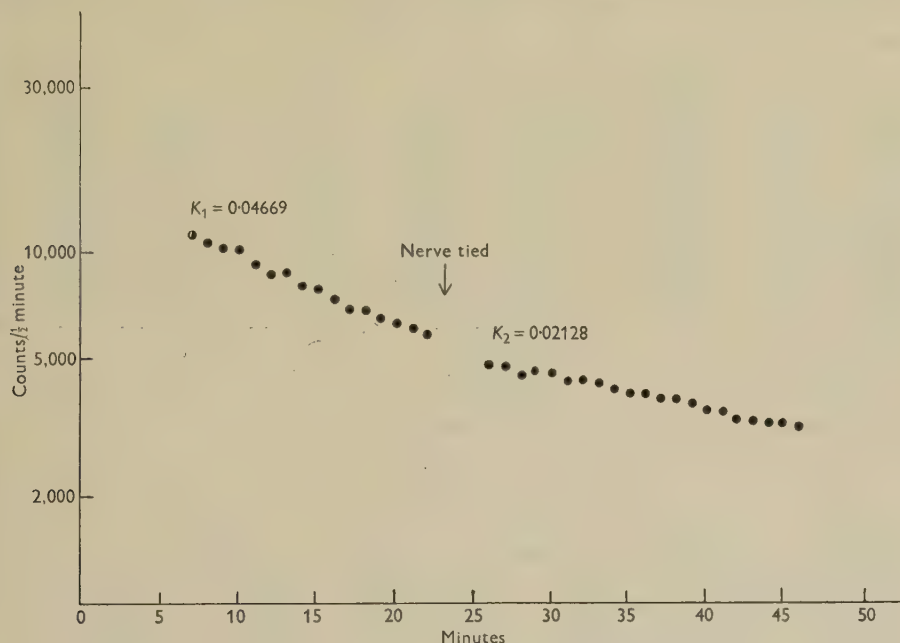


Fig. 3. Group 2, nerve tied inferiorly, Exp. 4.

Group 3. Ligature of the inferior group of vessels (Fig. 4, Table 1)

In this group of animals the vascular interruption consisted of ligature of the vessels supplying the nerve at its lower end. They were under-run with fine ophthalmic sutures on eyeless needles and separately ligated, each artery and vein together. An alteration in the mean initial clearance slope of 48.82% was found.

Group 4. Ligature of the nerve trunk superiorly (Fig. 5, Table 2)

The ligature in these experiments was placed immediately below the origin of the nerve to the hamstrings, and in such a way as to include the regional blood supply from the profunda vessels. The whole of the intrinsic and regional blood supply from above was thus occluded, leaving the nerve supplied only by intrinsic longitudinal vessels from below. The resulting clearance slope alteration for the whole group of experiments was 42.65%.

Group 5. *Ligature of the superior group of vessels* (Fig. 6, Table 2)

In these experiments, the branches of the inferior gluteal artery and the perforating branch of the profunda femoris to the nerve were ligated together with their accompanying veins. A mean clearance slope alteration of 31.14% resulted.

In one experiment an obviously anomalous result was obtained, the final clearance slope being greater than the initial clearance slope. The result has been ignored in the calculation of the mean change in clearance slope for the group.

Table 1

Group 1. Control					Group 2. Nerve tied inferiorly					Group 3. Vessels tied inferiorly				
Initial slope		Final slope		Slope change K_1-K_2	Initial slope		Final slope		Slope change K_1-K_2	Initial slope		Final slope		Slope change K_1-K_2
n	$ K_1 $	n	$ K_2 $		n	$ K_1 $	n	$ K_2 $		n	$ K_1 $	n	$ K_2 $	
17	0.01454	20	0.01653	-0.00199	15	0.02195	21	0.01044	+0.01151	17	0.03136	22	0.01564	+0.01572
14	0.04361	18	0.03785	+0.00576	18	0.05112	21	0.01622	+0.03490	16	0.07067	20	0.02998	+0.04069
15	0.01618	22	0.01800	-0.00182	18	0.03907	22	0.01619	+0.02288	17	0.04692	21	0.02946	+0.01746
18	0.03499	22	0.02623	+0.00876	16	0.04669	21	0.02128	+0.02541	17	0.03362	22	0.02084	+0.01278
15	0.04948	21	0.04883	+0.00065	18	0.03721	22	0.01736	+0.01985	17	0.05441	22	0.03684	+0.01757
16	0.05464	19	0.04395	+0.01069	20	0.04122	20	0.01345	+0.02777	15	0.04674	19	0.03007	+0.01667
15	0.03613	20	0.02864	+0.00749	20	0.05807	20	0.04322	+0.01485	20	0.07055	20	0.02920	+0.04135
16	0.07931	21	0.06291	+0.01640	20	0.05623	20	0.02093	+0.03530	20	0.08312	20	0.02130	+0.06182
18	0.03237	20	0.02401	+0.00836	20	0.02830	20	0.01236	+0.01594	20	0.05885	20	0.03452	+0.02433
17	0.05433	20	0.03875	+0.01558	20	0.07067	20	0.03703	+0.03364	20	0.05165	20	0.03258	+0.01907
Mean	0.04156	—	—	+0.00699	—	0.04505	—	—	+0.02421	—	0.05479	—	—	+0.02675
$\frac{\overline{K_1-K_2}}{-\overline{K_1}} \times 100 = 16.82$					$\frac{\overline{K_1-K_2}}{-\overline{K_1}} \times 100 = 53.74$					$\frac{\overline{K_1-K_2}}{-\overline{K_1}} \times 100 = 48.82$				

n = number of observations used in the calculation of each parameter.

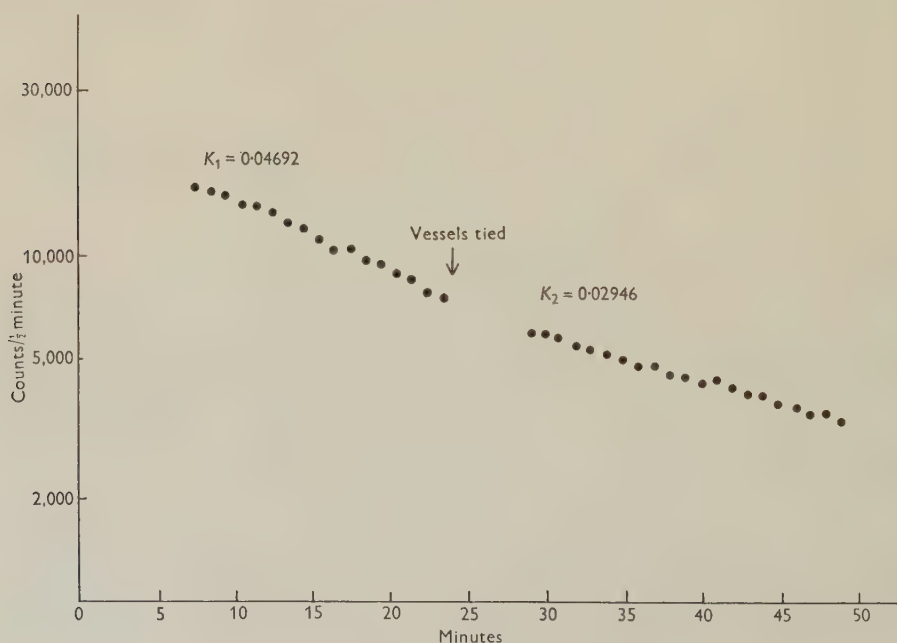


Fig. 4. Group 3, vessels tied inferiorly, Exp. 3.

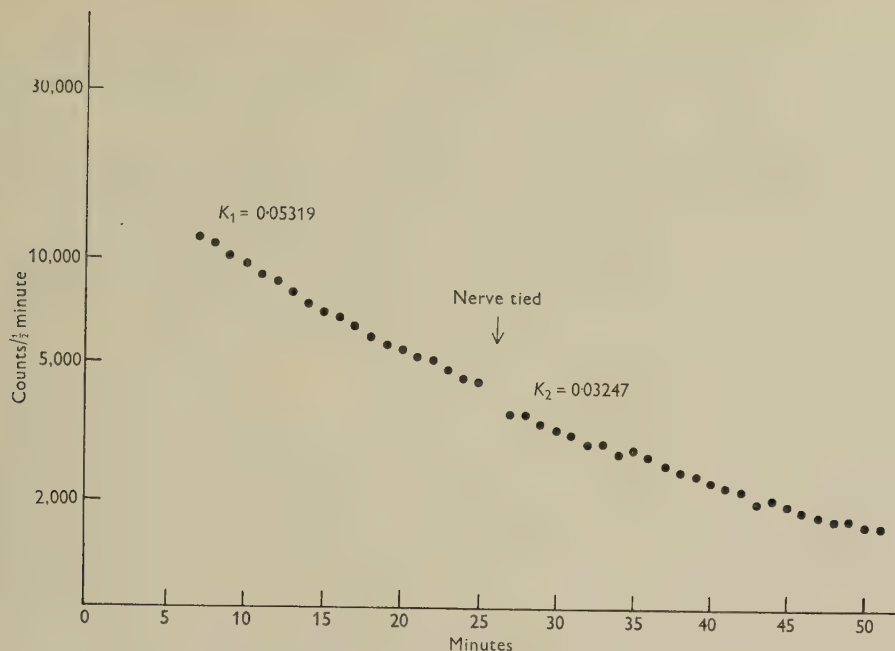


Fig. 5. Group 4, nerve tied superiorly, Exp. 3.

Table 2

Group 4. Nerve tied superiorly					Group 5. Vessels tied superiorly				
Initial slope		Final slope		Slope change $K_1 - K_3$	Initial slope		Final slope		Slope change $K_1 - K_2$
n	$ K_1 $	n	$ K_2 $		n	$ K_1 $	n	$ K_2 $	
16	0.03737	21	0.02657	+0.01080	16	0.04023	21	0.03180	+0.00843
18	0.06655	21	0.04301	+0.02345	16	0.04810	21	0.03318	+0.01492
19	0.05319	25	0.03247	+0.02072	16	0.04726	20	0.03774	+0.00952
16	0.01610	21	0.01177	+0.00433	17	0.03836	20	0.02560	+0.01276
17	0.03843	20	0.01950	+0.01893	15	0.03434	20	0.02452	+0.00982
17	0.06892	21	0.02973	+0.03919	16	0.02202	20	0.02687	-0.00485*
16	0.04713	21	0.01561	+0.03152	16	0.05150	20	0.03630	+0.01520
15	0.04420	20	0.03012	+0.01408	19	0.04587	20	0.02742	+0.01845
15	0.03662	20	0.02164	+0.01498	20	0.04398	20	0.02326	+0.02072
16	0.04750	20	0.02784	+0.01966	20	0.06705	20	0.04955	+0.01750
17	0.04767	20	0.03051	+0.01716†	20	0.04835	20	0.03088	+0.01747
Mean	0.04579	—	—	+0.01953	—	0.04650	—	—	+0.01448
$\frac{\overline{K_1 - K_2}}{-\overline{K_1}} \times 100 = 42.65$					$\frac{\overline{K_1 - K_2}}{-\overline{K_1}} \times 100 = 31.14$				

$$\frac{\overline{K_1 - K_2}}{-\overline{K_1}} \times 100 = 42.65$$

$$\frac{\overline{K_1 - K_2}}{-\overline{K_1}} \times 100 = 31.14$$

* Anomalous result in which final slope was higher than initial slope. Result has been ignored in the calculations.

† Experiment omitted by random selection from covariance analysis to equalize numbers in groups.

n = number of observations used in the calculation of each parameter.

DISCUSSION

Possible routes of clearance from the injection site

The nerve preparations used in the experiments were such that the only important route of clearance from the injection site was by way of vascular pathways. Diffusion along the length of the nerve and so out of the range of the counter, clearance into the surrounding tissue fluid, and direct clearance into the cerebro-spinal fluid were shown not to occur under the experimental conditions employed.

In regard to the findings on direct clearance to the cerebro-spinal fluid, it is suggested that the results differ from those of Brierley & Field (1950), who used

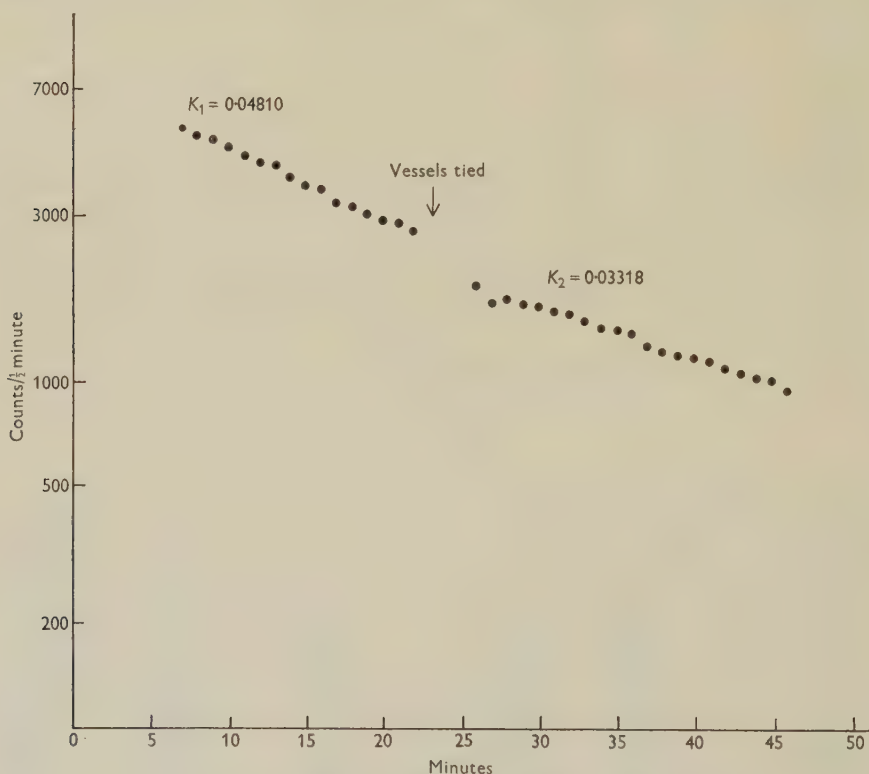


Fig. 6. Group 5, vessels tied superiorly, Exp. 2.

radioactive phosphorus, because of the small volume of fluid injected in the present experiments. It seems likely that larger volumes would be necessary to force the injected fluid sufficiently far along the intraneural spaces.

Injections were always made into the centre of the nerve trunk, and, since lymphatic channels have not yet been demonstrated in this situation the possibility of clearance by lymphatic channels would seem to be slight. Stone & Miller (1949) have, in fact, shown that the lymphatic system of the dog's leg does not play a significant role in the removal of intramuscularly injected radioactive sodium, a finding which might have been expected in view of the crystalloid nature of the

injection fluid. While the possibility of lymphatic clearance cannot be completely excluded, its presence or absence does not invalidate the results, which have been used only to compare the effects of different types of vascular occlusion. No absolute significance is claimed for the values obtained.

Control experiments

Exponential clearance of ^{24}Na injected into skin and muscle in various sites has been reported by numerous investigators, among whom are Kety (1949), Harris, Martin & Williams (1952), and Farmer (1953); indirect evidence has also been presented (Hyman, Rapaport, Saul & Morton, 1952; Walder, 1955) that blood flow is the most important factor in its removal, though other factors, among which the surface area and permeability of the capillary-tissue interface and the diffusion of the injected radio-sodium ions are perhaps the most important, must also be taken into consideration.

In preliminary experiments of the present series in which ^{24}Na clearance from the injection site was studied for uninterrupted periods of up to 70 mins., evidence of a gradual retardation in clearance was frequently noted, even after the observed counting rates were corrected for the background radioactivity and the paralysis time of the apparatus. This change in clearance was not found consistently, and could not be explained by the radioactive decay of ^{24}Na (half period 14.8 hr). The possibility of an additional exponential decay of radioactive phosphorus, occurring as a contaminant of the ^{24}Na has also been considered, but from data supplied by the Radiochemical Centre, Amersham, it is known that such contamination would account for less than 1 part in 1000 of the total radioactivity. It seems quite possible that the gradual retardation in clearance observed might be due, at least to some extent, to a gradual progressive alteration in the exposed vascular bed. Such a suggestion has been made by Buchanan, Walls & Williams (1954) to explain similar alterations in the clearance from skin. Perhaps, however, the most likely explanation in these experiments is that two different exponentials are involved in the clearance of radioactive sodium from mammalian nerve trunk. Dainty & Krnjević (1955) showed that the clearance from the nerve fibres was slower than that from the interstitial tissue fluid when *in vitro* experiments were carried out in a fluid medium. In the present series, by arbitrarily recording from two experimental periods of approximately 20 min., it was found that good correspondence to a linear semi-logarithmic curve could be obtained for each period. The effect of the radioactive decay of the ^{24}Na over such a period could be neglected, and it was possible to obtain an estimate from the slope change of not only the gradual retardation in clearance noted to occur over longer periods, but also of any effects on the vascular bed occasioned by removing a layer of waxed paper for use in checking the background radioactivity.

Differences in the general degree of vasodilatation in the exposed operative field between different animals were frequently observed. The initial clearance slope (K_1) and the final clearance slope (K_2) would be expected to be steeper in the presence of a generalized vasodilatation than from a more constricted vascular bed. In comparing the results in different groups of experiments covariance analysis has been used to make allowance for the variation in the initial slopes. Because of the clearance

slope changes noted to occur in the control series it is not possible to attach exact numerical significance to the percentage clearance slope alterations which were found in the experimental groups. However, the covariance analysis has shown that, in all cases except group 5 (nerve tied superiorly), the changes in clearance slope were significantly different from those obtained in the control series.

Differences between experimental groups

Ligation of the nerve trunk occluded both the regional vessels and the intrinsic vascular plexuses from one end of the sciatic nerve trunk, since the ligatures were placed between the injection site and the nearest regional vessels of supply. It has been found that at the lower end of the sciatic nerve the results of nerve ligation and of ligation of regional vessels alone are similar to one another, and covariance analysis reveals no significant difference between them. It may therefore be concluded that the major part of the increased clearance slope alteration obtained by nerve ligation (group 2) was due to the concomitant occlusion of the regional blood supply. The nerve therefore depends, for its blood supply at the lower end of the thigh, much more upon the regional vessels than upon its intrinsic longitudinal plexuses.

At the upper end of the thigh the regional vessels make a less substantial contribution to the blood supply of the nerve. It was found that ligation of the regional vessels (group 5) produced a much smaller proportionate reduction in clearance slope compared with that produced by nerve ligation (group 4) than was the case at the lower end of the thigh. Whereas in group 4 experiments the results differed significantly from the control series, the results of ligation of the regional vessels alone (group 5) did not so differ. Moreover, the results of nerve ligation and of ligation of the regional vessels differed significantly.

It seems likely that the difference in results between the upper and lower ends of the nerve can be explained by the anatomical arrangement of the regional vessels. Those at the upper end of the thigh are much closer to the numerous proximal vasa nervorum to the nerve and its roots within the pelvis than are those at the lower end of the thigh to the more distal vasa nervorum. The tibial nerve receives no regional vessels between the lower end of the thigh and the medial supramalleolar region, and, though the peroneal nerve is supplied by the anterior tibial artery, the branch thereto is small and very likely does not make any substantial contribution to the blood supply of the sciatic nerve trunk.

It will be noted that the results of both nerve ligation and of ligation of the vessels at the upper end of the thigh yield lower clearance slope changes than was the case in corresponding experiments at the lower end of the thigh. The nerve was injected halfway down the back of the thigh and this situation appeared to correspond to a point halfway between the entry of the vasa nervorum at the respective ends of the thigh. The regional blood supply from the profunda femoris, however, appears considerably more tenuous than is the case with the other regional vasa nervorum, and it seems likely that the injection point chosen was in fact somewhat nearer to the territory primarily subserved by the lower regional vessels, which would account for the difference in clearance slope alteration observed at the two ends of the nerve.

A predominance of the importance of regional blood supply, such as has been observed at the lower end of the rabbit sciatic nerve, is in contrast to the reported

findings of other investigations in which the regional vessels of supply to nerve trunks have been ligated in the course of recovery experiments. The most positive findings have come from the investigations of Denny-Brown & Brenner (1944) who reported patchy swelling of the axis cylinders with vacuolation of the myelin, which persisted for 12–14 days. Adams (1943), Bacsich & Wyburn (1945*a, b*) and Roberts (1948) obtained results of a relatively negative character, which might suggest that the initial predominance of regional blood supply, when present, is rapidly superseded after injury.

SUMMARY

The results of experiments, in which the clearance of radioactive sodium from the rabbit sciatic nerve was retarded by acute operative interventions on the blood supply of the nerve, are presented.

It has been shown that the vasa nervorum at the lower end of the thigh are more effective sources of blood supply, in acute experiments, than are the longitudinal intrinsic plexuses of the nerve trunk. This is not the case at the upper end of the thigh and the reasons for the differences observed have been discussed.

Warm thanks are expressed to Dr D. A. Sholl who has advised on the statistical treatment of the data, and to Mr G. Currie who has very kindly given much help with the calculation of the clearance slopes. We are grateful to Prof. R. E. M. Bowden and Dr H. A. B. Simons for encouragement and for their useful criticism. Mr H. S. Williams kindly arranged for the preparation of all the ^{24}Na , obtained as solid sodium chloride from A.E.R.E. Harwell, in a form suitable for use in the experiments.

Mr G. Champion has provided us with valuable technical assistance.

One of us (M.J.B.) is grateful to the Wellcome trustees for library facilities provided under a Wellcome Associateship of the Royal Society of Medicine during the period of this investigation.

REFERENCES

- ADAMS, W. E. (1943). The blood supply of nerves. *J. Anat., Lond.*, **77**, 243–250.
- BACSICH, P. & WYBURN, G. M. (1945*a*). The vascular pattern of peripheral nerve during repair after experimental crush injury. *J. Anat., Lond.*, **79**, 7–14.
- BACSICH, P. & WYBURN, G. M. (1945*b*). The effect of interference with the blood supply on the regeneration of peripheral nerves. *J. Anat., Lond.*, **79**, 74–82.
- BRIERLEY, J. B. & FIELD, E. J. (1950). The fate of an intraneural crystalloid injection as demonstrated by the radioactive tracer technique. *J. Anat., Lond.*, **84**, 61–62.
- BUCHANAN, T. J., WALLS, E. W. & WILLIAMS, E. S. (1954). Studies in radio-sodium clearance from the skin in man. *Clin. Sci.* **13**, 333–343.
- DAINTY, J. & KRNEVIĆ, K. (1955). The rate of exchange of ^{24}Na in cat nerves. *J. Physiol.* **128**, 489–503.
- DENNY-BROWN, D. & BRENNER, C. (1944). Paralysis of nerve induced by direct pressure and by tourniquet. *Arch. Neurol. Psychiat., Chicago*, **51**, 1–26.
- FARMER, F. T. (1953). Application of radio-isotopes as tracers in surgery. *Proc. R. Soc. Med.* **46**, 235–240.
- HARRIS, R., MARTIN, A. J. & WILLIAMS, H. S. (1952). Correlation of skin temperature and circulatory changes in muscle and subcutaneous tissue of the hand during trunk heating. *Clin. Sci.* **11**, 429–440.
- HYMAN, C., RAPAPORT, S. I., SAUL, A. M. & MORTON, M. E. (1952). Independence of capillary filtration and tissue clearance. *Amer. J. Physiol.* **168**, 674–679.

- KETY, S. S. (1949). Measurement of regional circulation by the local clearance of radioactive sodium. *Amer. Heart J.* **38**, 321-328.
- KOCH, E. (1926). Über den Einfluss vorübergehender Blutabspernung auf den Längsquerschnittstrom des Warmblutnerven. *Z. ges. exp. Med.* **50**, 238-257.
- OKADA, E. (1905). Experimentelle Untersuchungen über die vasculäre Trophik des peripheren Nerven. *Arb. neurol. Inst.* **12**, 59-85.
- ROBERTS, J. T. (1948). The effect of occlusive arterial diseases of the extremities on the blood supply of nerves. Experimental and clinical studies on the rôle of the vasa nervorum. *Amer. Heart J.* **35**, 369-392.
- SNEDECOR, G. W. (1946). *Statistical Methods*, 4th ed. p. 318. Iowa State College Press.
- STONE, P. W. & MILLER, W. B. (1949). Mobilization of radioactive sodium from the gastrocnemius muscle of the dog. *Proc. Soc. exp. Biol., N.Y.*, **71**, 529-534.
- UMBREIT, W. W., BURRIS, R. H. & STAUFFER, S. S. (1949). *Manometric Techniques and Tissue Metabolism*. Minneapolis.
- WALDER, D. N. (1955). The relationship between blood flow, capillary surface area and sodium clearance in muscle. *Clin. Sci.* **14**, 303-315.

THE FUNCTIONAL SIGNIFICANCE OF THE PATTERN OF INNERVATION OF THE MUSCLE QUADRATUS LABII SUPERIORIS OF THE RABBIT, CAT AND RAT

By RUTH E. M. BOWDEN AND Z. Y. MAHRAN*

Royal Free Hospital School of Medicine, London

INTRODUCTION

The proprioceptive supply of facial musculature has been the subject of many investigations from which conflicting conclusions have been drawn. Those who deny its existence are supported by the fact that spindles and other types of sensory endings have not yet been demonstrated in this group of muscles, which was studied by Cipollone (1896-8, 1897), Baum (1899), Smith (1926), Haggqvist (1938), and Bruesch (1944). However, in spite of these negative results, the pattern of innervation of facial muscles has been investigated in detail in the cat, rabbit and rat. Encouragement to do so came from the experience of Daniel (1946) and Cooper & Daniel (1949), for they succeeded in finding sensory endings in extrinsic ocular muscles of various species where other careful investigations had previously proved fruitless.

MATERIAL AND METHODS

Observations were made on muscles taken from six cats, six rats, twelve normal rabbits and seven rabbits sacrificed at varying times after cutting the seventh nerve immediately below the stylomastoid foramen. Four of these were sacrificed 7 days after operation, and the remaining three at 14, 21 and 31 days respectively. The animals were young healthy adults of both sexes and varying weights, and they were sacrificed by an overdose of ether.

Several different muscles were examined in the early stages of the investigation, but later attention was confined to the quadratus labii superioris, a compound muscle formed by the levators of the upper lip. This muscle was chosen because it was of appropriate size, shape and compactness for systematic histological examination, and its relation to the mystacial vibrissae suggested that it might be a suitable field in which to begin a search for proprioceptive endings.

For gold chloride preparations pieces of muscle were taken for immediate fixation from living anaesthetized animals. For all other stains fixation was carried out immediately after death by perfusing the animal with normal saline, followed by 10 % formol-saline. The fixed muscles were then dissected out whole and left for at least 10 days in 10 % formol-saline. Material from one rat and one rabbit received additional treatment with pyridine for 48 hr. The quadratus labii

* Present address: Anatomy Department, Abbassia Medical Faculty, Cairo. This work was done during study leave granted by the University of Ein Shams, Cairo, and it was presented in a Thesis on 'Innervation of facial musculature' for which the degree of Ph.D. was awarded by the University of London in 1955.

superioris of the cat and rabbit was cut into two or three segments before embedding in paraffin; the orientation of each piece was noted and preserved. Longitudinal serial sections were cut at 10–30 μ according to the stain used, and every fifth section was mounted. Frozen sections were also cut longitudinally at 15–40 μ .

STAINING TECHNIQUES

After experiment with the Gairns (1930) and Scott (1950) modifications of Ranvier's gold chloride impregnation, the following method was used, as results obtained with it proved more reliable in our hands:*

(1) *Fix and tease pieces of muscle (taken from the living anaesthetized animal) in 0.5 % formic acid for 30 min.*

(2) *Drain off fixative and remove excess with clean filter-paper.*

(3) *Stain for 60 min. in an aqueous solution of 1 % gold chloride (yellow or brown). This time was found to be critical.*

(4) *Remove excess stain with filter-paper.*

(5) *Place in 25 % formic acid for 4 hr. for limb muscles and up to 6 hr. for facial muscle. The fluid should be renewed if it becomes discoloured.*

(6) *Wash well in running tap water.*

(7) *Store in a cool place in equal parts of 95 % alcohol and pure glycerine.*

(8) *Tease and mount in pure glycerine.*

N.B. Stages 3 and 5 should be carried out in the dark with frequent shaking of the container, and all glass ware, including glass instruments, should be chemically clean.

Paraffin sections were stained with Romanes's (1950) and Glees's (1946) techniques, and frozen sections were stained by Glees's (1950) and Gros-Bielschowsky's methods.

OBSERVATIONS

GENERAL

The disposition of the fibres of quadratus labii superioris and the attachment of its fibres to the outer dermal sheath of the mystacial vibrissae is shown in Pl. 1, fig. 1 (cf. Scott, 1954). At the lower end its fibres intermingle with orbicularis oris and buccinator, and many of its fibres are attached to skin. These arrangements increased the difficulties of teasing the tissue and the vibrissae tended to break and distort paraffin sections. The intramuscular connective tissue also increased the difficulties of teasing and tended to take up the stains excessively. It was noticed that the rabbit's muscles contained relatively less connective tissue, and they responded more satisfactorily to the stains than those of the cat and rat. Of the silver stains, the Romanes method gave consistently good results and proved to be the best for serial sections; the other techniques gave some excellent results but their effects were erratic.

* Devised by R.E.M.B. when working on a Rockefeller Travelling Fellowship at the Poliomyelitis Research Center, Johns Hopkins University, Baltimore, Md., U.S.A., aided by a grant from the National Foundation for Infantile Paralysis Inc.

THE PATTERN OF INNERVATION

(1) *Nerve trunks, preterminal fibres, and motor end-plates*

The continuation of the upper buccal branch of the facial nerve ran deep to the postero-lateral border of the quadratus labii superioris in relation to its middle third. These branches communicated with those of the infra-orbital nerve and formed a plexus. Some of the branches of the infra-orbital nerve passed through the muscle on their way to supply skin and mucous membrane; the mystacial vibrissae were also supplied by its branches (Pl. 1, fig. 1).

Gold chloride preparations gave clear evidence of an intramuscular nerve plexus (Pl. 1, figs. 2, 3). In the three species the main intramuscular nerve trunks tended to run in a direction at right angles to the muscle fibres and they either gave off single preterminal fibres or bundles of a few preterminal fibres which formed a spray of endings (Pl. 1, figs. 4-6). In all preparations, and especially those stained with silver, many preterminal fibres were seen to run parallel with the muscle fibres (Pl. 1, figs. 6, 7). They either remained undivided, or, more frequently, divided into two at a variable, but short distance from the motor end-plate (Pl. 1, figs. 8, 9; Pl. 2, figs. 10, 11). These branches were usually of the same calibre, although this was not invariable (Pl. 2, fig. 12).

From examination of the muscle as a whole it was found that the motor end-plates were mainly confined to a broad band in the middle third of the muscle. In other regions they were few in number and in the part of the muscle which arose from bone no type of ending was found. In the gold chloride preparations the end-plates appeared to be more compact than in those stained with silver. In the latter many end-ramifications appeared to be elongated (cf. Pl. 1, fig. 9; Pl. 2, fig. 11). The two branches of a preterminal fibre either entered a single end-plate, or entered two discrete end-plates on a single muscle fibre (Pl. 1, fig. 9; Pl. 2, figs. 10, 12-14). Less frequently two separate end-plates on a single fibre were seen to be innervated by preterminal nerve fibres which could be traced to separate nerve bundles (Pl. 2, fig. 15). Pl. 2, fig. 16, shows a single end-plate supplied by two fibres of unequal thickness which approached from opposite directions. Pl. 2, figs. 17-19, also suggests that a single end-plate might be innervated by two axons, but it was not possible to exclude the division of a preterminal fibre at some distance from the muscle fibre. Three or even four end-plates were occasionally found on a muscle fibre (Pl. 2, fig. 14).

Both gold and silver staining showed that a variety of types of end-plate occurred in a single muscle, and there were no specific species differences in rabbit, cat and rat (Pl. 2, figs. 20-22). After section of the seventh nerve no innervated end-plates were found.

(2) *Innervation of blood vessels*

Perivascular nerve bundles and fibres were found in silver and gold-stained preparations made from normal and post-operative animals (Pl. 1, fig. 3; Pl. 3, fig. 23). Some of the fibres could be traced to their termination in the adventitia. After section of the seventh nerve intact nerve fibres were found in the perivascular

plexuses (Pl. 3, fig. 24), and some of these were seen to take part in the formation of the intramuscular plexus (Pl. 3, fig. 25). Others of these healthy perivascular nerves were traced back to the normal branches of the infra-orbital nerve.

(3) *Afferent innervation*

Four muscle spindles were identified in gold chloride preparations of a muscle taken from a single rabbit, which was the last of four animals which had been specially investigated for this purpose. In the four spindles it was possible to make out some of the features of the capsule, the intrafusal fibres and their innervation. In silver-stained preparations no spindles were found, but there was evidence of another type of sensory ending in one rabbit. No afferent endings were found in the cat and in the rat no spindles were found, but there was an unidentified type of ending; unfortunately no afferent endings were found in muscles taken from the post-operative animals.

(a) *Muscle spindles*

Capsule and intrafusal fibres. The capsule of the spindles was seen to be relatively thin as compared with that found in spindles of the limb muscles of the rabbit and rhesus monkey. In each of three spindles, two intrafusal muscle fibres could be identified, and these were of smaller diameter than the extrafusal fibres (Pl. 3, figs. 27-29). In these three the intrafusal fibres passed out of the capsule at the polar regions and intermingled with the extrafusal fibres. The fourth spindle was thin and appeared to contain only one intrafusal muscle fibre (Pl. 3, fig. 30).

The innervation of the spindle. The details of innervation of each spindle (I, II, III and IV) will be given separately. The pattern of innervation was less elaborate than that found in some spindles of the limb musculature in the rabbit (Barker, 1948). The latter type is comparable with the one shown in Pl. 3, fig. 26, taken from rhesus monkey.

(I) One axon penetrated the centre of the equatorial region (Pl. 3, fig. 27*a*) and another (Pl. 3, fig. 27*b*) entered the peripheral part of one polar region, ran for some distance and then divided into two. Each branch pursued a short and sinuous course, one ended in a simple knob and the other was broken off in its course towards the equatorial region. One axon entered what was presumed to be the other polar region, but it could not be followed to its termination, for a segment of this part of the spindle was broken off from the rest.

(II) In this spindle one axon entered the centre of the equatorial region (Pl. 3, fig. 28*a*) and another was seen to penetrate one of the polar regions and end immediately on one of the intrafusal muscle fibres (Pl. 3, fig. 28*b*). No axons were found entering the other polar region.

(III) Two axons entered the centre of the equatorial region of this spindle and a third, to one side of these, entered the myotube region of Barker (1948) (Pl. 3, fig. 29). Two axons were seen to enter one of the polar regions; one ended immediately in a knob on one of the intrafusal fibres and the other could not be followed. No axons could be found entering the other polar region.

(IV) No axons could be seen entering the equatorial region of this spindle but a flower-spray-like ending was found in this region (Pl. 3, fig. 30). A nerve fibre entered one of the polar regions and ran along the length of the spindle.

(b) *Spiral ending*

In a silver-stained preparation taken from another rabbit, a thick nerve fibre was seen to run parallel with a muscle fibre (Pl. 3, fig. 31). In addition to this axon, several finer nerve fibres formed a loose network along a length of the muscle fibre. No formed nerve endings were found in association with this network.

(c) *Unidentified endings*

There was a third type of ending which was only seen in the rat in sections stained by Romanes's technique. Thick preterminal fibres which might or might not first wrap round the muscle broke up into fine end-ramifications (Pl. 3, figs. 32-34). Small granules but no nuclei were visible in relation to the endings of these nerve fibres. An attempt was made to determine whether these endings were hypolemmal or epilemmal in position, but no definite conclusion was reached.

DISCUSSION

Although the scope of this investigation has been limited, it has raised several points of interest in the analysis of the functional organization of the peripheral nervous system. The features which merit special consideration are the presence of intramuscular nerve plexuses, the details of motor and vasomotor innervation and in particular the finding of spindles in the rabbit.

Feindel, Hinshaw & Weddell (1952) pointed out that the presence of an intramuscular plexus provided the anatomical pathway by means of which the branches of a single axon could innervate a number of scattered muscle fibres, but no direct histological evidence of this diffuse type of motor-unit was found in the present study. However, it has been shown that some perivascular nerves take a part in forming the plexuses, and it is possible that these plexuses might also be a means of achieving a final regrouping of the afferent and efferent nerve supply of the muscle itself. The intramuscular plexus would then be comparable with the fine intraneural plexuses which were described by O'Connell (1936). It is perhaps significant that sensory endings have now been found in most, but not all, of the muscles in which plexuses have been described.

The pattern of motor innervation raises the question of the innervation ratio and of the size of the motor-unit (Bors, 1926; Cooper, 1929; Eccles & Sherrington, 1930; Clark, 1931). When a visual comparison was made between serial sections of the human medial rectus muscle (known to have a high density of innervation, Bors, 1926), and the rabbit's quadratus labii superioris, there was a striking difference. In the medial rectus muscle each section showed numerous nerve fibres in every field (Pl. 3, fig. 35), whereas they were most numerous in the middle part and apparently absent from the upper part of the quadratus labii superioris. This difference does not necessarily indicate a contrast in degree of innervation, for it might reflect differences in the location of motor and sensory endings. In both

muscles the motor end-plates are confined to the middle portions, but in the medial rectus and indeed in the other human extrinsic ocular muscles both extremities contain numerous sensory endings (Cooper & Daniel, 1949; Merrillees, Sunderland & Hayhow, 1950). The nerve to the medial rectus enters at a single point on the ocular surface (Wolff, 1933), and its fibres would necessarily spread throughout the muscle to reach all the end-organs. The position of sensory endings in the quadratus labii superioris is as yet unknown, and therefore any qualitative comparison is inevitably misleading. It is obvious that the average size of the motor-unit can only be computed in a deafferented sympathetomized muscle.

An electromyographical study of the facial muscles in cat, rabbit and rat would be of interest as a supplement to histological investigation, for in man it has provided evidence which suggests that these muscles have diffuse and small motor-units (Weddell, Feinstein & Pattle, 1944; Feinstein, 1946; Jasper & Ballem, 1949; Petersén & Kugelberg, 1949; Bowden, 1954; Buchthal & Rosenfalck, 1955).

Parallel arrangement of preterminal fibres and the presence of more than one ending on a single muscle fibre has already been reported by Huber (1899) and Hines (1931) in extrinsic ocular muscles of the rabbit; Woollard (1931) in the same muscles in man, dog, cat and rabbit; and by Feindel *et al.* (1952) in extrinsic ocular muscles of man and monkey. Haggqvist (1938, 1940) stated that in facial muscles of the cat and monkey he could only find *en grappe* endings. These appeared to be identical with the complex endings described here in rabbit, cat and rat. The alinement of the preterminal fibres may be related to the fascicular architecture which is similar in these muscles, but the presence of multiple endings supplied by one axon is less easy to interpret. Huber (1899) came to the conclusion that they were sensory endings for several reasons: first, he was unable to identify end-plate nuclei in their vicinity; secondly, he found that they stained more rapidly with methylene blue than the classical type of motor ending; and thirdly, they appeared to be epilemmal structures. Hines (1931) and Woollard (1931) tentatively suggested that they were sensory organs, but Woollard found them in both epi- and hypolemmal positions. Haggqvist (1938, 1940) attributed a tonic function to them, and Feindel *et al.* (1952) identified them as a number of motor end-plates in series, although they felt unable to interpret their functional significance. The findings in this investigation support Feindel *et al.*, for here the endings were hypolemmal and end-plate nuclei were demonstrated unequivocally by the silver stains. This arrangement of end-plates in series would seem to be a means of increasing the area of contact between nerve endings and the subneural apparatus of Couteaux (1947); and the same result would be achieved by the more elaborate types of ramification in the single end-plates (Pl. 2, figs. 20*b*, 21*b*, 22*b*).

When a single muscle fibre has two or more motor end-plates which are innervated by axons approaching from different directions and different bundles (Pl. 2, fig. 15) appearances suggest, at first sight, that this one fibre may take part in two different patterns of motor activity. However, this hypothesis must remain unproven until the axons can either be traced back to separate motor neurones or proved to be of distinct origin by electro-physiological tests. Histological examination of the muscle is insufficient, for motor nerves branch in the peripheral nerve trunk, in the branches which enter the muscle belly and within the muscle itself

(Eccles & Sherrington, 1930). This branching, and the presence of intraneural and intramuscular plexuses, might allow branches of a single neurone to approach one muscle fibre from divergent directions.

The functional significance of a single end-plate which is supplied by two axons coming from two apparently different directions is obscure, particularly when one of the fibres is finer than the other (Pl. 2, fig. 16). From the evidence reviewed by Tiegs (1953), the fine fibre is unlikely to be autonomic in origin, but it might perhaps be one of the regenerated 'ultraterminal' fibres described by Hoffman (1951). These muscles are in an exposed position and might therefore be subjected to repeated minor traumata associated with local partial denervation followed by this type of local regeneration. However, no evidence of denervation was found in any of the unoperated animals and similar appearances have been seen in the well-protected extrinsic ocular muscles.

Huber's (1930) suggestion that fibres from the fifth nerve which communicated with the seventh nerve trunk might not only travel to the skin and its appendages, but also might supply the blood vessels has been confirmed here, and perivascular fibres have been seen to take a part in the formation of the intramuscular nerve plexuses. However, until section of the facial nerve is combined with a cervical sympathectomy it will not be possible to determine whether these fibres are afferents arising in the trigeminal nerve or sympathetic fibres which are distributed by its branches.

The last, and perhaps most important, point for discussion is the finding of sensory endings. In view of the experience of all those workers who have investigated extrinsic ocular muscles of various species during the last 70 years (see Cooper & Daniel, 1949), it is not remarkable that no sensory endings have yet been found in facial muscles, for up to the present there have been only a few investigations on normal foetal and adult facial muscles. It is still less surprising that Bruesch (1944) was unsuccessful when he searched in denervated muscles, where the difficulties are enhanced by atrophy, intramuscular fibrosis and alteration in staining reactions. So far only four spindles and one spiral ending have been found in the rabbit, and it will be necessary to count and plot the position of sensory endings in the quadratus labii superioris (cf. Cooper & Daniel, 1949; Merrillees, Sunderland & Hayhow, 1950) as a preliminary to an analysis of their innervation. It is not yet clear whether these proprioceptive endings are supplied exclusively by the facial or trigeminal nerves, or perhaps by both together (Mahran, 1955).

Although spindles have been found in the quadratus labii superioris of the rabbit it does not necessarily indicate that they will be found in other facial muscles of this animal, or that they will be found in muscles of other species. For example, Cooper & Daniel (1949) were unable to find them in the extrinsic ocular muscles of the rhesus macaque, dog, bear, cheetah, cat and rabbit. However, their explanation of this negative result is open to criticism and further search cannot be considered unnecessary. Although it is unwise to speculate on the presence or absence of sensory endings in facial muscles of an animal such as the cat, which has a relatively immobile face excepting in rage, it seems likely that they will be found in facial muscles of primates where the range and variety of movements are much greater than in the rabbit, for there is increasing evidence of the important part played by afferent impulses in the organization of patterns of motor activity.

The capsule of the spindles found in *quadratus labii superioris* appeared to be simpler than in some of those found in limb muscles of the rabbit (cf. Barker, 1948) and rhesus monkey (Bowden, unpublished) (Pl. 3, fig. 26). Cooper & Daniel (1949) observed a difference in the thickness of the capsule in spindles of the extrinsic ocular muscles and lumbricals in man, and put forward two hypotheses to account for this finding. In the first it was suggested that as the capsule was part of a recording mechanism, its thickness might be related to the threshold of response to the stretch stimulus, and therefore presumably it would be thin where the threshold was low and thick where it was high. In the second hypothesis a protective function was suggested, for the capsule was found to be thin in the *erector spinae* where the spindles were in the depth of the muscle, and it was thick in the lumbricals. The observations in this present investigation support the first hypothesis, for the *quadratus labii superioris* is inserted into the skin of an exposed and relatively unprotected part, and in fact the lumbrical muscles are protected by overlying tendons, the dense palmar aponeurosis, fat and thick palmar skin.

The pattern of innervation of the spindles described here is simple, and resembles the more simple type of spindle found by Barker (1948) in the rabbit's limb muscles. The absence of motor innervation at one pole of spindles II and III may have been fortuitous and due to hazards of teasing the tissue. An interesting feature is seen in fig. 27 (Pl. 3) for the nerve fibre which entered the polar region, and was therefore presumably a motor fibre, passed up to the equatorial region in which sensory fibres are found (Barker, 1948), and in Pl. 3, fig. 30, one fibre entered at one pole and ran the whole length of the single intrafusal fibre.

The spirally arranged network of nerve fibres which was found in another rabbit was considered to be a sensory ending, for it was epilemmal in position, resembling the endings found by Daniel (1946) in human extrinsic ocular muscles. On the other hand, as it was not possible to be certain whether the endings found in the rat were epilemmal or hypolemmal in position, no definite function can be assigned to them yet, although they bear a morphological resemblance to some of the sensory endings described by Daniel (1946). It might be suggested that they were artefacts due to faulty staining of motor end-plates, but this seems unlikely for there were classical motor end-plates in the same sections which were well and evenly stained.

SUMMARY AND CONCLUSIONS

1. The facial muscles, and in particular *quadratus labii superioris*, have been examined by various histological techniques in six cats, six rats, twelve normal rabbits, and seven rabbits after section of the right facial nerve below the stylo-mastoid foramen at varying intervals after operation.
2. The arrangement of intramuscular nerve trunks and perivascular plexuses and the morphology of motor endings have been described in detail.
3. Muscle spindles have been found in one rabbit and a spiral sensory ending was found in another one.
4. No sensory endings were found in the cat.
5. Unidentified endings were found in the rat.
6. The functional significance of these observations has been discussed.

It is a pleasure to record our grateful thanks to the Medical Faculty of the University of Ein Shams, Cairo, for study leave awarded to Z. Y. M., and for a generous contribution towards the cost of the photography; to Messrs J. M. Crane and E. V. Willmott and Mrs P. Thomas for their skill and patience in producing the photomicrographs; to the Augustus and Alice Waller Research Trust for some of the photographic apparatus; and lastly, but not least, to our colleagues for helpful discussion and criticisms.

REFERENCES

- BARKER, D. (1948). The innervation of the muscle spindle. *Quart. J. micr. Sci.* **89**, 143-186.
- BAUM, J. (1899). Cited by Hines (1930). Beiträge zur Kenntnis der Muskelspindeln. Wiesbaden: J. F. Bergmann. (Thesis unobtainable.)
- BORS, E. (1926). Über das Zehnenverhältnis Zwischen Nerven-und Muskelfasern. *Anat. Anz.* **60**, 415-416.
- BOWDEN, R. E. M. (1954). Peripheral nerve injuries. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 282, p. 276. H.M. Stationery Office.
- BRUESCH, S. R. (1944). The distribution of myelinated afferent fibers in the branches of the cat's facial nerve. *J. comp. Neurol.* **81**, 169-191.
- BUCHTHAL, F. & ROSENFALCK, P. (1955). Action potential parameters in different human muscles. *Acta psychiat., Kbh.*, **30**, 126-131.
- CIPOLLONE, L. T. (1896-8). Cited by Hines (1930). Nuove ricerche sul fuso neuro-muscolare. *Ric. Lab. Anat. norm. Univ. Roma*, **6**, 157-200.
- CIPOLLONE, L. T. (1897). Cited by Hines (1930). Ricerche sull'anatomia normale e pathologica delle terminazioni nervose nei muscoli striati. *Ann. Med. nav. colon.* (Suppl.), **3**, 1-282.
- CLARK, D. A. (1931). Muscle counts of motor units: a study of innervation ratios. *Amer. J. Physiol.* **96**, 296-304.
- COOPER, S. (1929). Relation of active to inactive fibres in fractional contraction of muscle. *J. Physiol.* **67**, 1-13.
- COOPER, S. & DANIEL, P. M. (1949). Muscle spindles in human extrinsic eye muscles. *Brain*, **72**, 1-24.
- COUTEAUX, R. (1947). Contribution à l'étude de la synapse myoneurale, p. 633. Thèses présentées à la Faculté des Sciences de l'Université de Paris. Montréal: Thérien Frères, Limitée.
- DANIEL, P. M. (1946). Spiral nerve endings in extrinsic eye muscles of man. *J. Anat., Lond.*, **80**, 189-193.
- ECCLES, J. C. & SHERRINGTON, C. S. (1930). Numbers and contraction-values of individual motor-units examined in some muscles of the limb. *Proc. Roy. Soc. B*, **106**, 326-357.
- FEINDEL, W., HINSHAW, J. R. & WEDDELL, G. (1952). The pattern of motor innervation in mammalian striated muscle. *J. Anat., Lond.*, **86**, 35-48.
- FEINSTEIN, B. (1946). The application of electromyography to affections of the facial and the intrinsic laryngeal muscles. *Proc. R. Soc. Med.* **39**, 817.
- GAIRNS, F. W. (1930). A modified gold chloride method for the demonstration of nerve endings. *Quart. J. micr. Sci.* **74**, 151-154.
- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath.* **5**, 54-59.
- GLEES, P. (1950). In Weddell, G. & Zander, E. A critical evaluation of methods used to demonstrate the tissue neural elements, illustrated by reference to the cornea. *J. Anat., Lond.*, **84**, 178.
- HAGGQVIST, G. (1938). Zur Kenntnis einer doppelten cerebrospinalen Innervation der Skelettmuskeln. *Z. mikr.-anat. Forsch.* **43**, 491-508.
- HAGGQVIST, G. (1940). A contribution to the question of the nervous and muscular substratum of the muscle tone. *Acta med. scand.* **104**, 8-20.
- HINES, M. (1930). The innervation of the muscle-spindle. *Res. Publ. Ass. nerv. ment. Dis.* **9**, 124-152.
- HINES, M. (1931). Studies on the innervation of skeletal muscle. III. Innervation of the extrinsic eye muscles of the rabbit. *Amer. J. Anat.* **47**, 1-54.

- HOFFMAN, H. (1951). Fate of interrupted nerve fibres regenerating into partially denervated muscles. *Aust. J. exp. Biol. med. Sci.* **29**, 211–219.
- HUBER, E. (1930). Evolution of facial musculature and cutaneous field of trigeminus. *Quart. Rev. Biol.* **5**, 133–188, 389–437.
- HUBER, G. C. (1899). A note on sensory nerve-endings in the extrinsic eye muscles of the rabbit. 'Atypical motor endings of Retzius'. *Anat. Anz.* **15**, 335–342.
- JASPER, H. & BALLEM, G. (1949). Unipolar electromyograms of normal and denervated human muscle. *J. Neurophysiol.* **12**, 231–244.
- MAHRAN, Z. Y. (1955). Innervation of facial musculature. Thesis approved for award of degree of Ph.D., Univ. London.
- MERRILLEES, N. C. R., SUNDERLAND, S. & HAYHOW, W. (1950). Neuromuscular spindles in the extraocular muscles in man. *Anat. Rec.* **108**, 23–30.
- O'CONNELL, J. E. A. (1936). The intraneural plexus and its significance. *J. Anat., Lond.*, **70**, 468–497.
- PETERSÉN, I. & KUGELBERG, E. (1949). Duration and form of action potential in the normal human muscle. *J. Neurol. Psychiat.* **12**, 124–128.
- ROMANES, G. J. (1950). The staining of nerve fibres in paraffin sections with silver. *J. Anat., Lond.*, **84**, 104–115.
- SCOTT, M. G. A. D. (1954). Distribution, structure, development and mode of action of vibrissae of some mammals. Thesis approved for award of degree of M.Sc., Univ. London.
- SCOTT, P. P. (1950). The use of hyaluronidase in microtechnique. *Nature, Lond.*, **166**, 479.
- SMITH, O. C. (1926). Personal communication to Hines, M. (1930).
- TIEGS, O. W. (1953). Innervation of voluntary muscle. *Physiol. Rev.* **33**, 90–144.
- WEDDELL, G., FEINSTEIN, B. & PATTLE, R. E. (1944). The electrical activity of voluntary muscle in man under normal and pathological conditions. *Brain*, **67**, 178–257.
- WOLFF, E. (1933). *The Anatomy of the Eye and Orbit*, p. 136. London: H. K. Lewis.
- WOOLLARD, H. H. (1931). The innervation of the ocular muscles. *J. Anat., Lond.*, **65**, 215–223.

EXPLANATION OF PLATES

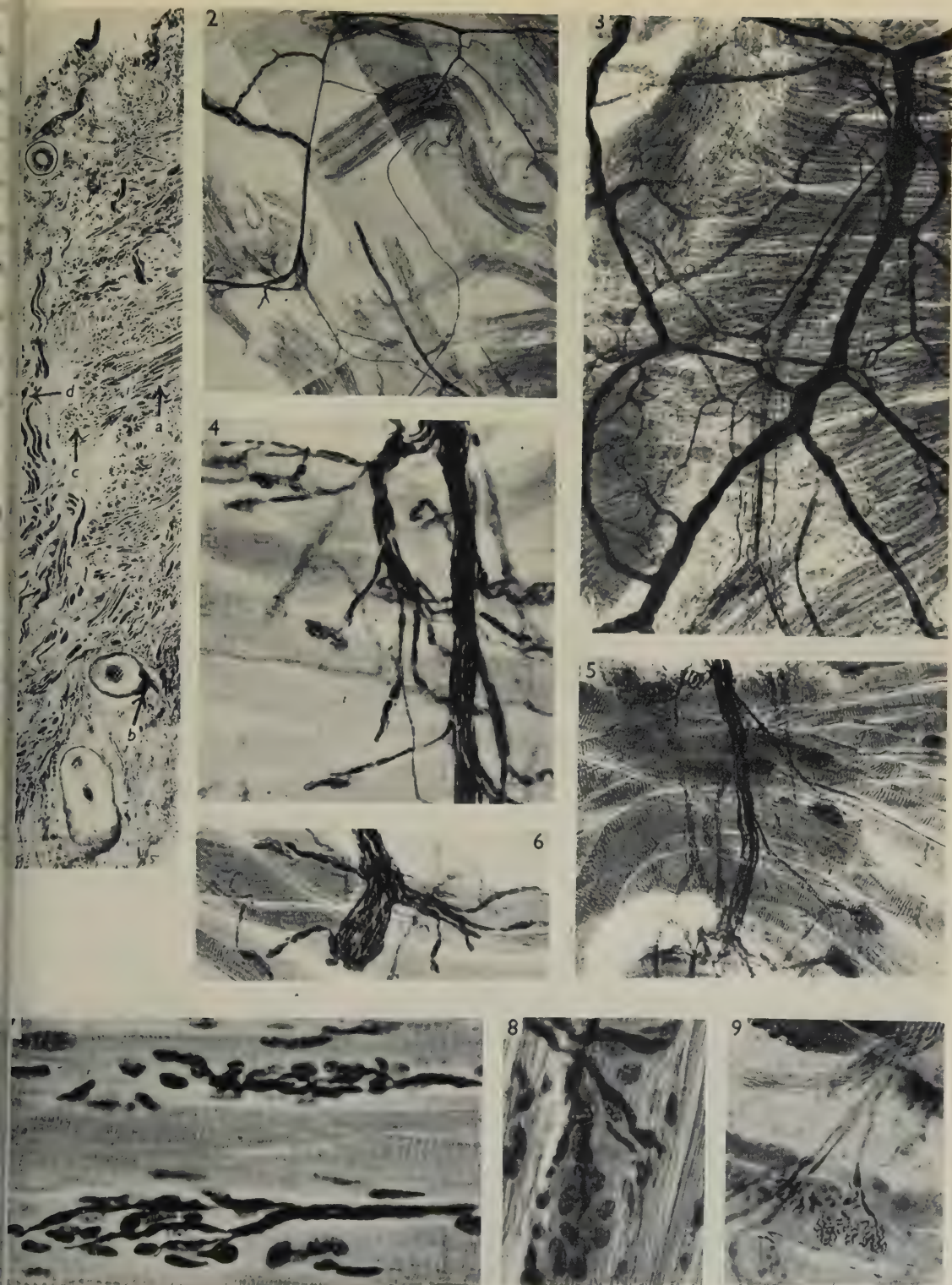
All illustrations shown here are of m. quadratus labii superioris (m.q.l.s.). Unless otherwise stated, they are prepared from the rabbit.

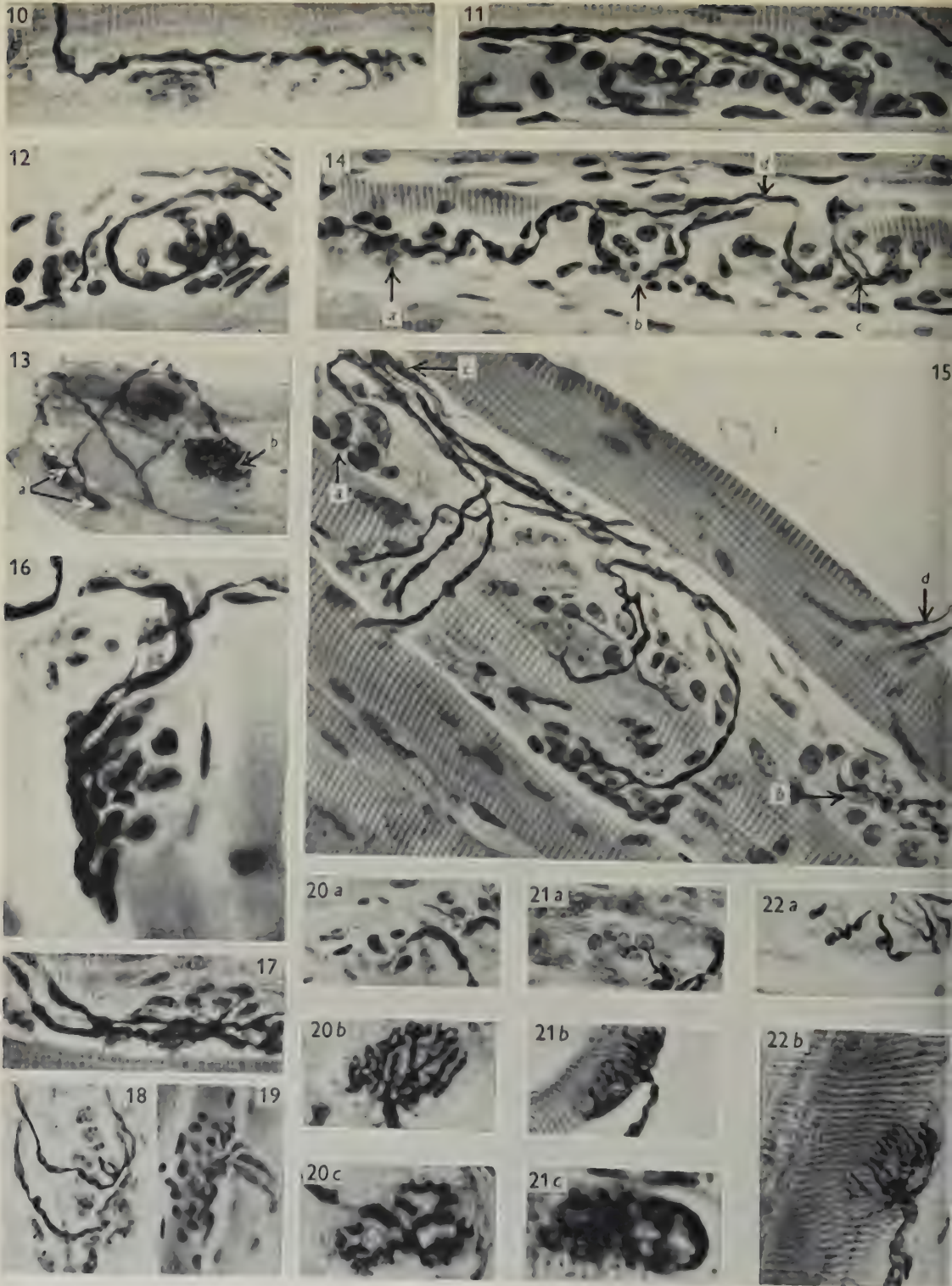
PLATE 1

- Fig. 1. Section through rt. upper lip of rabbit 7 days after operative division of the rt. facial nerve. Note the fibres of m.q.l.s. (a) which are passing towards their insertion into skin and into the theca of the vibrissae (b). The fibres of orbicularis oris are cut in T.S. (c). The intact nerve fibres (d) are from the infra-orbital branches of the trigeminal nerve. Romanes's stain, $\times 7.5$.
- Fig. 2. Intramuscular plexus of nerves. Gold chloride stain, $\times 14$.
- Fig. 3. Intramuscular plexus: note nerve fibres contributing to the perivascular plexus. Gold chloride stain, $\times 53$.
- Figs. 4–6. Intramuscular nerve trunk and motor endings. Rabbit, cat and rat respectively. Gold chloride stain, $\times 150$.
- Fig. 7. Preterminal nerve fibres lying parallel with muscle fibres. Note the branching of the lower fibre within the end-plate. Romanes's stain, $\times 560$.
- Fig. 8. Unbranched nerve fibre entering motor end-plate. Glees's stain, $\times 400$.
- Fig. 9. Nerve fibre dividing into two branches a short distance before entering a motor end-plate. Gold chloride, $\times 200$.

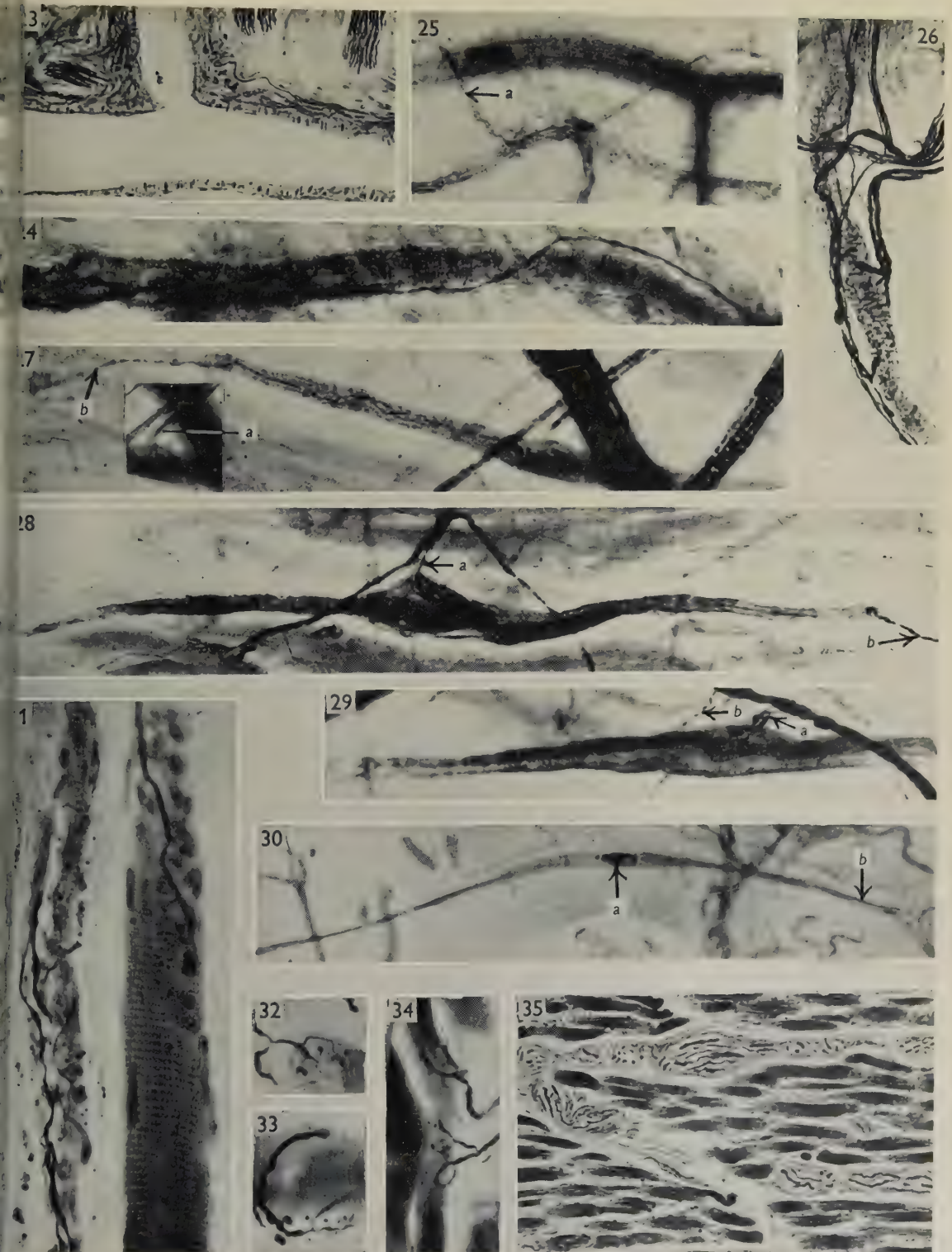
PLATE 2

- Fig. 10. Two separate motor end-plates on a single muscle fibre, supplied by branches of one nerve fibre. Romanes's stain, $\times 560$.
- Fig. 11. Elongated motor end-plate supplied by two branches of a single nerve fibre. Romanes's stain, $\times 560$.
- Fig. 12. Nerve fibre dividing into two branches of unequal calibre to supply separate contiguous motor end-plates on a single muscle fibre. Romanes's stain, $\times 600$.
- Fig. 13. (a) Two discrete motor endings on a single muscle fibre supplied by the same axon. (b) Single end-plate supplied by an axon which divides into two just before entering the end-plate. Gold chloride, $\times 200$.





BOWDEN AND MAHRAN—MUSCLE QUADRATUS LABII SUPERIORIS OF THE RABBIT, CAT AND RAT



- Fig. 14. Three motor end-plates (*a*, *b* and *c*) on one muscle fibre; *a* and *b* are innervated by the same axon which divides at *d*. Romanes's stain, $\times 480$.
- Fig. 15. Two motor end-plates (*a* and *b*) on a single muscle fibre, but innervated by axons derived from different bundles (*c* and *d*) which approach from different directions. Romanes's stain, $\times 560$.
- Fig. 16. Single motor end-plate supplied by axons of unequal calibre which approach from opposite directions. Romanes's stain, $\times 840$.
- Figs. 17, 18. Motor end-plates apparently supplied by two nerve fibres. Romanes's stain, $\times 560$.
- Fig. 19. Motor end-plate apparently supplied by two nerve fibres. Romanes's stain, $\times 400$.
- Fig. 20. Examples of types of motor ending in rabbit. (*a*) Romanes's stain, $\times 400$. (*b*) and (*c*) Gold chloride, $\times 400$.
- Fig. 21. Examples of types of motor ending in cat. (*a*) Glees's stain, $\times 400$. (*b*) and (*c*) Gold chloride, $\times 400$.
- Fig. 22. Examples of types of motor ending in rat. (*a*) Romanes's stain, $\times 400$. (*b*) Gold chloride, $\times 400$.

PLATE 3

- Fig. 23. Perivascular nerve bundles and fibres. Romanes's stain, $\times 150$.
- Fig. 24. Intact perivascular nerve fibre 14 days after section of the facial nerve. Gold chloride, $\times 160$.
- Fig. 25. Two intact nerve fibres (*a*) which enter a degenerating bundle of the facial nerve, leave almost immediately to form part of a perivascular plexus. Gold chloride, $\times 70$.
- Fig. 26. Spindle from sartorius muscle of rhesus macacus. Gold chloride, $\times 100$.
- Fig. 27. (*a*) Axon entering equatorial region of spindle, $\times 100$. (*b*) Axon entering polar region and dividing into two branches, $\times 115$. Gold chloride.
- Fig. 28. (*a*) Axon entering equatorial region of spindle and dividing into two. (*b*) Axon entering polar region and forming a simple ending on one of two intrafusal fibres. Gold chloride, $\times 100$.
- Fig. 29. Spindle with two intrafusal fibres. (*a*) Two axons entering the centre of the equatorial region. (*b*) A third axon entering the myotube region. Gold chloride, $\times 100$.
- Fig. 30. Spindle consisting of a single intrafusal fibre. Note flower-spray-like ending (*a*) in the equatorial region; a single axon (*b*) which enters one pole. The latter was traced along the length of the spindle. Gold chloride, $\times 100$.
- Fig. 31. Spiral-ending with a thick nerve fibre running parallel with the muscle fibre and a network of fine nerve fibres around the muscle fibre. Note motor end-plate on right. Romanes's stain, $\times 560$.
- Figs. 32-34. Unidentified endings in rat. Romanes's stain, $\times 400$.
- Fig. 35. Post-mortem specimen of human extrinsic ocular muscle (rectus medialis) showing numerous nerve bundles and fibres. Romanes's stain, $\times 100$.

OBSERVATIONS ON THE SILVER IMPREGNATION OF NERVE FIBRES IN TEETH

BY R. W. FEARNHEAD AND J. E. LINDER

Departments of Anatomy and Dental Histology, The London Hospital Medical College

It is widely accepted that small beaded nerve fibrils form a marginal plexus in the predentine parallel to the pulpo-dentinal surface. Whether or not branches from the marginal plexus continue into the calcified dentine, following an intratubular course (Tojoda, 1934), or are situated in the dentine matrix, is uncertain. To examine this problem it was decided to make, in the first instance, a carefully controlled investigation into the method of impregnating nerves in teeth with silver, taking into account Samuel's (1953*a, b*) recent evaluation of the many factors which influence the mechanism of silver impregnation. In view of the observations made by Weddell, Pallie & Palmer (1954), use was also made of the *in vivo* and *in vitro* methylene blue methods (Schabadasch, 1935) as a control for the results of the impregnation with silver.

MATERIAL AND METHODS

Formalin-fixed 10 μ paraffin sections of decalcified human teeth, and of the pulps removed from human premolars and molars, were used for the staining tests. The method of staining was based on that described by Holmes (1943), and was chosen because each stage of the procedure could be adapted for experimental purposes and tested separately.

The deparaffinized sections were washed thoroughly in running tap water to remove traces of formalin or acid, followed by three changes of glass-distilled water, and buffered overnight (approx. 16 hr.) at 37° C. The sections were then transferred to fresh buffer solution containing 1 ml. of 1 % solution of silver nitrate to every 100 ml. of buffer. The sections were left in this solution in the dark for periods varying from 1 to 10 hr. After this initial impregnation, the sections were rinsed *quickly* in distilled water and transferred to 2.5 % sodium sulphite for periods varying from 1 to 10 min. After sulphite treatment the sections were washed in running tap water, followed by three changes of distilled water and then placed in the developing solution. Development was carried out for 3 min. at 29° C. in a mixture used by Pearson & O'Neill (1946), which consisted of 3 % gelatine, 2 % silver nitrate, 1 % hydroquinone in the proportions of 4 : 2 : 1. After development, sections were washed thoroughly in distilled water, toned with 0.2 % gold chloride for 10 min, and washed in distilled water. The gold was reduced with 2 % oxalic acid for 5 min. or with 1 : 10,000 resorcinol for 30 min. Sections were then washed and fixed in 5 % sodium thiosulphate, and finally washed, dehydrated and mounted in neutral canada balsam. The stages in what is at present employed as the standard technique for sections of teeth are shown in Text-fig. 1, which also indicates the various points which were found to have profound effects on the impregnation.

RESULTS

In evaluating the results it was borne in mind that any method should possess the following properties:

- (1) The impregnation should demonstrate completely all the nervous elements present in the dental tissues.
- (2) The impregnation should show clearly the morphology of the smallest axons.
- (3) The impregnated nerves should be well differentiated from other tissue elements.
- (4) The results should be repeatable with certainty on routinely prepared dental tissues.

Silver technique adopted after experimentation

- | | |
|--|---|
| 1. Washing deparaffinized sections | ← { 2 hr. in running tap water for formalin fixed tissues
16-18 hr. in running tap water for other fixatives,
Bouin, Rossman, etc. |
| ↓ | |
| 2. Three changes of distilled water | ← 1 hr. each change—the last change in double glass-distilled water |
| ↓ | |
| 3. Overnight 12-14 hr. in concentrated Palitzsch boric acid borax buffer pH 7.0 maintained at 37° C. | ← Mix twice amount required, use half for initial buffering and remainder for silver impregnation |
| ↓ | |
| 4. 2 hr. in 1 : 10,000 silver nitrate in Palitzsch buffer pH 7.0 maintained at 37° C. | ← When transferring to silver impregnating solution agitate sections to remove bubbles which may otherwise adhere to section and interfere with the impregnation. |
| ↓ | |
| 5. Rinse quickly in double-distilled water | |
| ↓ | |
| 6. Place in 2.5 % sodium sulphite for 5 min. at room temperature 20° C. | ← Mix the developer at this point and maintain at 29° C. in a water-bath for the next 14 min. |
| ↓ | |
| 7. Wash in running tap water for 4 min. | |
| ↓ | |
| 8. Distilled water for 10 min. | ← Three changes, double glass-distilled |
| ↓ | |
| 9. Developer for 3 min. at 29° C. | |
| ↓ | |
| 10. Wash in distilled water | ← Until all trace of developing solution is removed |
| ↓ | |
| 11. Tone in 0.2 % gold chloride for 10 min. | |
| ↓ | |
| 12. Wash 1 min. in distilled water | |
| ↓ | |
| 13. Reduce gold with 1 : 10,000 resorcinol in distilled water for 30 min. | ← The time taken for the reduction of the gold may be decreased by using a stronger solution of resorcinol |
| ↓ | |
| 14. Wash in distilled water | |
| ↓ | |
| 15. 5 min. in 5 % sodium thiosulphate | |
| ↓ | |
| 16. Wash, dehydrate, clear and mount | |

Text-fig. 1

It will be seen from the following that the factors which have the greatest influence on the impregnation are simple and obvious ones, but inadequate control of any one of them may spoil the resulting impregnation. Our early results were inconsistent, some sections giving an excellent staining (Pl. 1, fig. 1 *a-c*), some showing a good impregnation of nerves spoiled by a very heavy staining of other tissues (Pl. 1, fig. 2), and in some an indiscriminate staining of non-nervous elements (Pl. 1,

fig. 3). These results, at first very puzzling, became more comprehensible as each factor was evaluated.

Washing

Preliminary washing in running tap water for 2 hr. was found to be the shortest time adequate for sections of formalin-fixed material. Tissues fixed in Bouin, Zenker or Rossman fluids required from 12 to 18 hr. washing in order to remove completely all traces of salts and metals which might either react with the silver or cause local fluctuation in the pH. In all, thirty-eight different fixatives have been tested, of which Bouin's fluid seemed to be the most promising. The results of the series of experiments described here, however, were obtained on formalin-fixed material.

Buffer

The Palitzsch buffer solutions were prepared with 19.0715 g. $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ /litre of double glass-distilled water for solution A and 12.386 g. H_2BO_3 /litre for solution B, weighing error being within ± 0.1 mg. It is common practice to use buffer solution diluted 1 in 10. At this dilution it was thought that at the extreme ends of the pH range covered by the solution the buffering capacity might be reduced. Solutions A and B were mixed and checked with electrodes on a direct reading pH meter to give a pH reading of 6.8, diluted 1:10 with double glass-distilled water (pH 7.0) and tested again at room temperature 20°C ., at 37° and 53.5°C . At this dilution, at 20°C ., the pH of the diluted buffer slowly rose from 6.8 to 7.2 over a period of 30 min. At 37°C . a similar rise in pH occurred but more rapidly, and at 53.5°C . even more rapidly. Concentrated buffer solution, mixed to give a pH 6.8, maintained this pH even when the temperature was raised. Concentrated buffer solutions with and without silver or sections were maintained at 37°C . over a period of 24 hr. without any change in the pH occurring. Maintaining sections at this temperature fits into routine laboratory work very well, and the use of concentrated Palitzsch buffer for this technique is recommended.

Because of the dense nature of the dentine matrix, a considerable time must be allowed for the complete penetration of the section by the buffer solution. For this reason sections were allowed to remain in buffer overnight in a thermostatically controlled oven at 37°C . In practice it was found convenient to prepare twice the amount of buffer required to cover the sections and to use half for overnight buffering and the remainder for the actual silver impregnation.

Using sections of sympathetic ganglia and spinal cord from rabbits fixed in a special fixing solution (Davenport & Kline, 1938), Samuel (1953*a*) has shown that within limits there is an inter-relationship between temperature, pH, silver nitrate concentration and time of the impregnation. Sections of tooth pulps were therefore impregnated for increasing periods from 1 to 10 hr. in 1/10,000 buffered silver nitrate solution maintained at 37°C . over a pH range of 6.2, 6.4, 6.8, 7.0, 7.4 and 7.9. In order to maintain the pH at 6.2 an acetic acid-sodium acetate buffer was used (Pearse, 1953), Palitzsch buffer being used for the remainder. The results supported the observations of Samuel (1953*a*). The optimal time of impregnation found to be necessary, however, was always longer than in his experiments which he carried out at 53.5°C ., but this was probably due to the lower temperature during impregnation. It was found very difficult to assess within fine limits the optimal conditions of

impregnation, because at the time these experiments were carried out, control over development had not been obtained. Pl. 1, figs. 4–6, show sections impregnated at pH 6·8 for 6 hr., pH 7·0 and 7·4 for 2 hr. Impregnation at pH 7·0 and 7·4 appeared to give the best results. Even at pH 6·2 an impregnation of nervous elements was obtained, however, but only after prolonged immersion in the silver solution. In sections impregnated for short periods at this pH the quality of the impregnation was poorly defined and granular (Pl. 1, fig. 7). In all later experiments, therefore, the silver impregnation solution was buffered to pH 7·0, and the section impregnated for 2 hr. at 37° C.

Sulphite treatment

After the initial impregnation, sections were immersed in 2·5 % sodium sulphite solution for 5 min. to remove the reducible silver from the non-nervous tissues in the sections. It was found that if sections were left longer than this the silver was progressively removed, first from the finest nerve fibrils and later from nuclei and larger nerves. If sections were left for long periods in distilled water instead of the sulphite solution the silver was removed from the sections in a similar manner but more slowly. Removal of silver by distilled water was accelerated if the sections were first treated for a short time with sulphite. It was also found that sections could be left in running tap water for considerable periods without apparent loss of silver. Furthermore, removal of silver from the section did not occur if they were placed in distilled water after the tap-water treatment. This effect is probably due to the precipitation of the silver by impurities in the tap water, but the exact nature of these impurities has not been elucidated. It was found, however, that the loss of silver from sections could be prevented by placing them in a solution of 0·1 g. of sodium chloride per litre of glass-distilled water instead of tap water. Impurities in tap water are known to vary considerably from place to place, and may well play a more important role in successful silver impregnation than has hitherto been generally accepted. As a result of these observations, after immersion in the sodium sulphite bath for 5 min., sections were washed in running tap water for 4 min., and then transferred to distilled water for 10 min. prior to development.

Physical development

A greatly improved impregnation of the nerves was repeatedly obtained under the modified conditions already described, but differentiation was poor, and nerves and nuclei appeared densely black on a pale background of connective tissue which contained granular deposits of silver in the cytoplasm. It was thought that some improvement might result if greater control over the developing technique could be achieved.

It was noticed that the solution used for physical development exhibited striking changes during the first few minutes after mixing, when the opalescent straw colour of the freshly mixed solution changed rapidly to a deep amber and finally to an opaque 'muddy' grey-green fluid. In view of these observations it was necessary to decide whether to place impregnated sections in the freshly mixed physical developer, to wait until the clear amber stage was reached or to start development when the mixture had reached the 'muddy' stage. A series of impregnated sections were therefore immersed in the developing solution at intervals increasing by 3 min.

increments from the time of mixing the developer. Each section was allowed to remain in the developer for 3 min. The total time covered by the experiment was 27 min. In addition, in order to eliminate the possibility of the developing power of the solution being reduced by the successive development of sections, the experiment was repeated using a separate pot for each section. In both series the cleanest impregnation, with good nuclear detail and well-differentiated background, was obtained in sections developed 12 or 15 min. after mixing the developer.

Gold toning

It was believed that a slower rate of reduction of the gold chloride during toning might result in a deposit of gold of finer grain size in the tissues and thus enhance the colour differentiation between nervous and non-nervous elements. Among the many substances tried, reduction with 1 : 1100 aniline water for 30 min. or 1 : 10,000 resorcinol for 30 min. appeared to give results superior to those obtained with oxalic acid, a substance more commonly used in gold toning techniques. The longer time taken for the toning proved to be an advantage since the progress of differentiation could be watched and checked under the microscope (Pl. 1, figs. 8-10).

DISCUSSION

Weddell & Zander (1950) have made a critical evaluation of methods for the demonstration of peripheral sensory nerves. They compared the results given by silver, methylene blue and phase-contrast techniques with the appearance of living nerves in the cornea viewed by the slit lamp and corneal microscope. They came to the conclusion that methylene-blue techniques gave the most accurate representation of axis cylinder in stained preparations. A series of specimens of rat skin, and the pulps of teeth from the cat, goat and man were therefore prepared using the methylene-blue method described by Schabadasch (1935) for comparison with the results of our silver experiments. The fine beaded terminal filaments could be seen clearly in both methylene blue and silver preparations, and the vesicular character of some of the larger fibres was clearly demonstrated by the silver sections (Pl. 1, figs. 11, 12).

Recently, Weddell & Pallie (1954) have shown that even in the most carefully fixed tissue distortion takes place sufficient to confuse the interpretation of sections prepared with both methylene blue and silver methods. They have found that a great deal of this distortion can be avoided and more rapid fixation obtained by subcutaneous injection of hyaluronidase a short time before introducing the dyes or fixatives. Use of hyaluronidase with this technique was not included in our experiments, but since the rate of penetration of dyes or fixatives is extremely slow in dentine, future investigations along these lines might prove very profitable.

On decalcified tissues the results of the silver technique described were consistent and gave a clear and well differentiated impregnation of the nerves. The nerve plexus described by Raschkow (1835) was well demonstrated, and very thin branches could be easily traced from it passing between the odontoblasts. Loops of fine nerves in the predentine as described by Bradlaw (1939) were clearly seen, and, in addition, beaded fibres almost at the limit of optical resolution could be traced forming a subdental or marginal pulp plexus. Numerous tiny branches could be traced

entering the dentinal tubules, many of them passing a considerable distance into the tubules well past the zone of predentine.

Some criticism of the use of physical development methods in the demonstration of nerve fibres has been made by Romanes (1950) on the basis that the nerve fibres are made to appear larger than they are in fact. Fernández-Morán (1952) has described the presence of nerve fibres of submicroscopic dimension in the central nervous system, and Robertis & Sotelo (1952) have described fine processes which are below the limits of optical resolution at the tips of neurites grown in tissue culture. It seems, therefore, not impossible that submicroscopic fibres could exist as fine prolongations of nerves within the dentinal tubules in teeth. If this should be so, to increase the diameter of such fibres so that they come within the resolving power of the light microscope would appear to be a justifiable technique.

SUMMARY

An attempt has been made to evaluate the factors which may influence the silver impregnation of nerve fibres in teeth. The method of impregnation was based on that described by Holmes (1943). It was found that insufficient control over any one of the factors summarized below could prevent the successful impregnation of nerve fibres in teeth.

1. Inadequate removal of fixatives during the preliminary washing of deparaffinized sections.
2. Instability of the pH of the impregnating solution (even more important than the pH level chosen), although neutral or alkaline pH gave better results than sections buffered on the acid side of neutrality.
3. Impurities in tap water have an important role in preventing the loss of too much silver from the sections after treatment with sodium sulphite solution.
4. The developing solution used in this technique rapidly undergoes striking changes after mixing; consequently there is a critical period during which development of impregnated sections should be commenced. This period was found to be 12–15 min. after the time of mixing when the temperature of the developing solution is 29° C.
5. More delicate differentiation between nerves and non-nervous elements was obtained after toning with gold chloride if the gold was reduced with aniline or resorcinol instead of oxalic acid.

The standardized technique was found to give consistently a reproducible impregnation of nerve fibrils in decalcified sections of teeth. By comparison with preparations of nerves stained by *in vivo* and *in vitro* methylene blue methods, the silver technique was regarded as giving a reliable histological representation of the nervous elements in teeth.

We are deeply indebted to Prof. A. E. W. Miles and Prof. R. J. Harrison for their many helpful suggestions, and to Mr A. L. Gallup for printing the photomicrographs. The expenses incurred by this study were defrayed by a grant to one of us from The Yarrow Research Fund, The London Hospital Medical College.

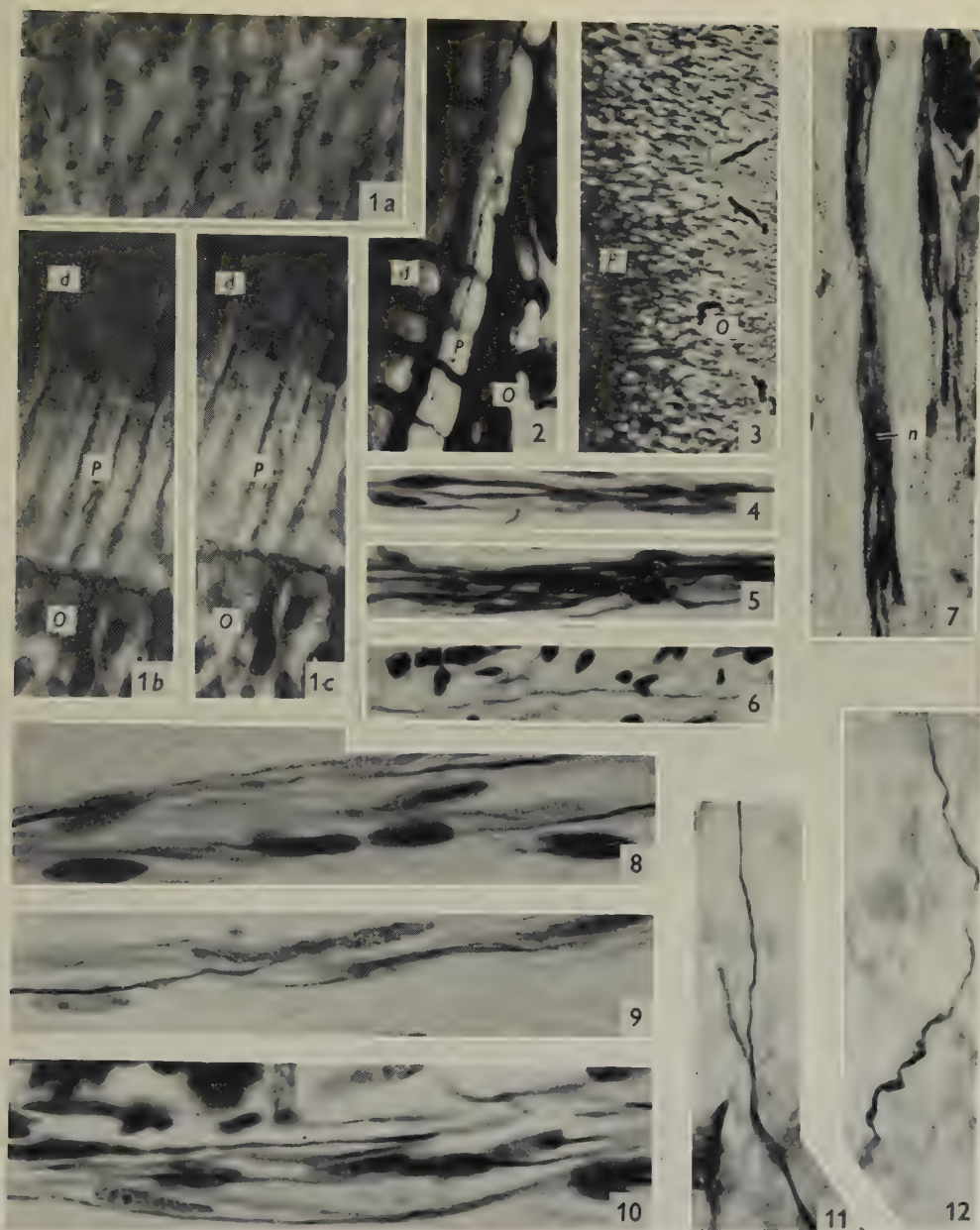
REFERENCES

- BRADLAW, R. (1939). The histology and histopathology of the dental innervation. *Proc. R. Soc. Med.* **32**, 1040–1053. (Sect. Odontol., pp. 68–81).
- DAVENPORT, H. A. & KLINE, C. L. (1938). Part 2. Use of *n*-propyl and *n*-butyl alcohol in Hofker's fixative. *Stain Tech.* **13**, 147–160.
- FERNÁNDEZ-MORÁN, H. (1952). The submicroscopic organization of vertebrate nerve fibres. *Exp. cell. Res.* **3**, 5–83.
- HOLMES, W. (1943). Silver staining of nerve axons in paraffin sections. *Anat. Rec.* **86**, 157–187.
- PEARSE, A. G. E. (1953). *Histochemistry, Theoretical and Applied*. London: J. and A. Churchill Ltd.
- PEARSON, A. A. & O'NEILL, S. L. (1946). A silver-gelatin method for staining nerve fibres. *Anat. Rec.* **95**, 297–301.
- RASCHKOW, J. (1835). *Die Gesundheitslehre des Mundes*. Ludwigsburg.
- ROBERTIS, E. & SOTELO, J. R. (1952). Electron microscope study of cultured nervous tissue. *Exp. cell Res.* **3**, 433–452.
- ROMANES, G. J. (1950). The staining of nerve fibres in paraffin sections with silver. *J. Anat., Lond.*, **84**, 104–115.
- SAMUEL, E. P. (1953*a*). Impregnation and development in silver staining. *J. Anat., Lond.*, **87**, 268–277.
- SAMUEL, E. P. (1953*b*). The mechanism of silver staining. *J. Anat., Lond.*, **87**, 278–287.
- SCHABADASCH, A. (1935). Theoretische und experimentelle Studien zur Methylenblaufärbung des Nervengewebes. *Acta morphologia*, H. 1. Staatsverlag, Gorkij. (Cited by Romeis, B. (1948). *Mikroskopische Technik*, Leibniz Verlag, München.)
- TOJODA, M. (1934). Die Innervation des menschlichen Zahnbeins, *Dtsch. Zahnärztl. Wchschr.* **37**, 641–645, 670–673.
- WEDDELL, G. & PALLIE, W. (1954). The value of 'spreading' factors in the demonstration of tissue neural elements. *Quart. J. micr. Sci.* **95**, 389–397.
- WEDDELL, G., PALLIE, W. & PALMER, E. (1954). The morphology of peripheral nerve terminations in the skin. *Quart. J. micr. Sci.* **95**, 483–501.
- WEDDELL, G. & ZANDER, E. (1950). A critical evaluation of methods used to demonstrate tissue neural elements, illustrated by reference to the cornea. *J. Anat., Lond.*, **84**, 168–195.

EXPLANATION OF PLATE

Abbreviations: *O* = odontoblasts; *P* = predentine zone; *d* = dentine; *n* = nerve.

- Fig. 1 (*a*). Small beaded nerve fibres passing between the odontoblast processes close to the pulpal surface of the predentine. Tangential section through the pulpo-dentinal junction of a human premolar. Formalin fixation, decalcified, silver impregnation, 2 hr. at pH 7·0, gold toned/resorcinol reduction. $\times 1000$. (*b*) Small beaded nerve crossing the pulpo-predentinal junction, close to an odontoblast process and passing into a tubule in the predentine. Human premolar, formalin fixation, decalcified, silver impregnation, 2 hr. at pH 7·0, gold toned/resorcinol reduction. $\times 800$. (*c*) The same field as (*b*). Difference in focus level approximately 0·5 μ .
- Fig. 2. A small beaded nerve situated in the predentine, notice however that the preparation is spoiled by a very heavy deposit of silver in the dentinal tubules and the odontoblasts. Human premolar, formalin fixation, decalcified, silver impregnation, 2 hr. at pH 7·0, Palitzsch buffer diluted 10 ml. buffer : 100 ml. impregnating solution (washed 2 hr. running tap water, buffered for only 2 hr. before impregnation) untuned. $\times 800$.
- Fig. 3. Silver impregnated connective tissue fibres at the pulpo-predentinal junction. Human premolar, decalcified, silver impregnation, 2 hr. at pH 7·0, Palitzsch buffer diluted 10 ml. buffer : 100 ml. impregnating solution (washed 2 hr. in running tap water, placed in buffer solution only 2 hr. before impregnation) untuned. $\times 90$.
- Figs. 4–10 are photomicrographs of paraffin sections of the pulp removed from an undecalcified human premolar after formalin fixation. Sections illustrated in Figs. 4–7 are from series impregnated in 1 : 10,000 silver nitrate. The variable factors were pH and time of impregnation. They were then developed in freshly mixed 'physical developer' and the gold toning reduced with 2% oxalic acid.



FEARNHEAD AND LINDER—SILVER IMPREGNATION OF NERVE FIBRES IN TEETH

(Facing p. 234)

- Fig. 4. 6 hr. impregnation at 37° C. with dilute silver solution buffered to pH 6.8. Gold toned/oxalic acid reduction. $\times 390$.
- Fig. 5. 2 hr. impregnation at 37° C. with dilute silver nitrate solution buffered to pH 7.0. Gold toned/oxalic acid reduction. $\times 390$.
- Fig. 6. 2 hr. impregnation at 37° C. with dilute silver solution buffered to pH 7.4. Gold toned/oxalic acid reduction. $\times 390$.
- Fig. 7. 10 hr. impregnation at 37° C. with dilute silver solution buffered to pH 6.2. Gold toned/oxalic acid reduction. $\times 390$.
- Sections in figs. 8–10 were impregnated for 2 hr with 1 : 10,000 silver nitrate solution, buffered to pH 7.0 and development commenced 15 min. after mixing the developer, but the method of gold toning was varied.
- Fig. 8. Gold toning reduced with 2% oxalic acid for 5 min. $\times 1200$.
- Fig. 9. Gold toning reduced with 1 : 1000 aniline water for 30 min. $\times 1200$.
- Fig. 10. Gold toning reduced with 1 : 10,000 resorcinol for 30 min. $\times 1200$.
- Fig. 11. Nerves in pulp of a human molar formalin fixed, decalcified, silver impregnation, 2 hr. at pH 7.0. $\times 1200$.
- Fig. 12. Nerve in the pulp of human premolar. Methylene blue (Schabadasch) *in vitro* preparation. $\times 820$.

OBSERVATIONS ON THE POSTURAL MECHANISM OF THE HUMAN KNEE JOINT

BY J. W. SMITH

*Department of Anatomy, St Salvador's College,
University of St Andrews*

INTRODUCTION

An essential factor in the maintenance of the normal postures of standing (Smith, 1953) is the temporary stabilization of the joints transmitting body weight. It is widely held that the knee joint, in common with other weight-bearing joints, is orientated and stabilized by that low grade and diffuse firing of the antigravitational muscles which has been called postural or tonic contraction. This view is based on the classical work of Sherrington, Magnus and their co-workers, and involves a transference of the results of their animal experiments to the postural mechanism of man.

In the experimental animal the position of the knee joint is controlled during standing by the postural contraction of the quadriceps muscle, but this fact, of itself, gives no clue to the identity of the antigravitational muscles at the human knee joint: this can only be determined by a consideration of the influence exerted by body weight on that joint during standing. In the child who is learning to stand, the angle between the leg and thigh is considerably less than 180° (Pl. 1, fig. 3), and it is apparent that body weight tends to flex the knee joint. Thus in the infant, as in the experimental animal, the muscles which are potentially capable of effecting stability at the knee joint during standing are the extensor group. On the other hand, in the stance of the adolescent and adult, as is now generally accepted (Johnston & Whillis, 1954; Brash, 1951), the centre of gravity of the body falls in front of the axes of the knee joints, and the angle between the leg and thigh exceeds 180° (Pl. 1, figs. 4, 5). Consequently, in these age groups, the quadriceps muscle cannot control the position of the knee joint as has been suggested by Denny-Brown (1949): on the contrary, the ability to perform this function is necessarily transferred to the flexor muscle group. The conditions existing at the adult human knee joint appear to be peculiar to man, indeed, there is considerable evidence to suggest that they are peculiar to modern man (Morton, 1935; Hooton, 1947), and it might therefore be postulated that the ontogenetic change in the posture of the knee joint during standing, and consequently in the identity of the antigravitational muscles, is a recapitulation of a phylogenetic change.

The electromyographic studies of the adult lower limb muscles in standing which have been described by Seyffarth (1940, 1941), Weddell, Feinstein & Pattle (1944), Joseph & Nightingale (1952), Smith (1954*a*), indicate that there is in fact a low-grade activity in the hamstring and posterior crural muscle groups. Attempts to compute the total muscle force represented by such electromyograms are notoriously hazardous, though it has been suggested recently (Lippold, 1952) that it can be accomplished with some accuracy in the case of voluntary contraction in the living subject. In

any event, it seems probable that the electromyographic record of the flexor muscles during standing represents an activity insufficient of itself to stabilize the knee joint. As a result there is to-day increasing support (Wright, 1952; Ralston & Libet, 1953) for the view that the adult knee joint is stabilized during standing by two complementary and harmonious factors. One of these factors is the postural contraction of the flexor muscles, and the other is the passive resistance of the tissues of the part. This view has been clearly expressed by Clemmesen (1951) thus: 'The relaxed standing position... is determined and equilibrium obtained by postural reflex tone in association with the elastic forces and in opposition to gravity.'

The investigation described in this paper is an inquiry into the nature of the *passive* mechanism which resists extension at the knee joint, and the extent of the contribution which it makes to the stabilization of the knee joint in various postural circumstances.

It is postulated at the outset that the passive resistance to extension at the knee joint is divisible into two components which normally operate in harmony with each other. The one component is the resistance which is exerted by the recognized articular mechanism described by Goodsir (1868), and this will be referred to as the articular resistance; the other component is that exerted by all those extra-articular structures which are stretched by the movement of extension, and this will be referred to as the extra-articular resistance.

Before proceeding to an experimental analysis of these two factors, a description is given of the elastic properties of the articular and extra-articular tissues, and of the influence of these properties on the nature of the resistance.

The nature of the articular resistance

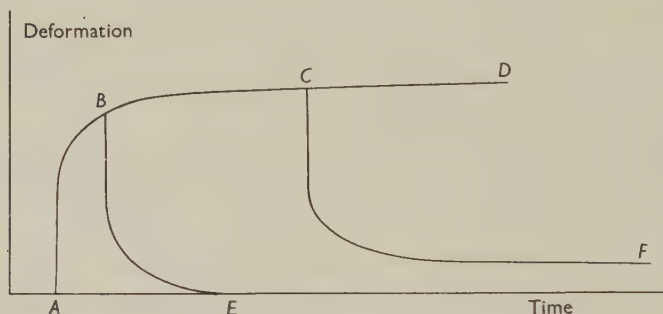
The articular mechanism which limits extension at the knee joint was described by Goodsir (1868), and this description has been elaborated and clarified by the recent work of Barnett (1953). Goodsir showed that in osteo-ligamentous preparations of the knee joint, extension is limited by a mechanism which involves the tightening of joint ligaments, and the simultaneous locking of paired articular surfaces of logarithmic spiral curvature; that is, by tensile stresses in joint ligaments and compression stresses in the tissues deep to the articular surfaces. It is widely held that the tissues involved in this mechanism, namely joint ligaments, articular cartilage, and intra-articular fibrocartilages, are practically unchanged in their dimensions by any external force to which they might normally be subjected. Moreover, this conception has led to the assumption that Goodsir's mechanism becomes operative at one particular position of the knee joint, and at that position arrests extension immediately and completely, an assumption which is in conformity with the view of Walmsley (1928).

If this were the true nature of the locking mechanism at the knee joint, then it would have to be accepted that only in exceptional circumstances would the mechanism be a significant factor in the stabilization of the knee joint, for in all common activities, such as standing, walking and running, the maximum extension of that joint is always appreciably short of the full voluntary movement (see below).

However, there is now abundant evidence that the tissues involved in the articular mechanism, far from being rigid, are each appreciably distorted by stress

of normal dimensions. Furthermore, within certain limits, each of these tissues reacts elastically when relieved of stress.

The ready deformability and elasticity of articular cartilage has long been recognized (Braune & Fischer, 1891). Bär (1926) and, more recently, Hirsch (1944) recognized that the elasticity of this tissue has peculiar features: when it is subjected, after death, to a uniform compression stress, instantaneous deformation is followed by a finite rate of deformation which becomes less as the duration of the load is prolonged. When the stress ceases, recoil follows a similar pattern (Text-fig.1), but the extent of recoil, i.e. the elasticity, varies with the duration of the preceding stress. After stress of short duration (less than 1 min.) elasticity is perfect, but with stresses of longer duration the elasticity becomes increasingly imperfect. Ingelmark & Ekholm (1948) confirmed these reactions and proposed on experimental evidence that they were due to a dual mechanism of compression and recoil. They suggested that both processes were due in part to true elasticity (instantaneous deformation and recoil) and in part to a migration of fluid between the cartilage on the one hand



Text-fig. 1. The deformation of articular cartilage caused by a constant compression stress is shown by a line *ABCD*. If the stress ceases after a short time the recoil *BE* is complete, but if the stress is maintained until *C*, the recoil *CF* is incomplete.

and the synovial fluid and the vessels of the marrow cavity on the other. It seems probable, therefore, that the imperfect elasticity noted by Bär and Hirsch is in some measure at least due to the lack, under experimental conditions, of the fluid pool on which cartilage must draw during recoil, and that during life a relatively prolonged compression stress would be followed by a recoil which, although gradual, was eventually complete. At all events it is obvious that in life articular cartilage must normally recoil completely after stress of physiological dimensions, and Fairbank (1948) has demonstrated that the recoil is in fact delayed, so that the articular cartilage of the knee joint is some 2 mm. thinner in the evening than it is in the morning, and recovers its original thickness only after a period of rest. Thus in relation to physiological stress, living articular cartilage is readily deformable and perfectly elastic, but both the extent of the deformation and the duration of the process of recoil are dependent on the magnitude, the duration and the proximity in time of that stress.

It is also worthy of note that as a result of these observations Ingelmark (1950) has put forward the hypothesis that it is by the migration of fluid, brought about by repeated discrete compression stresses, that the nutrition of articular cartilage

is maintained. A corollary to this is, that there must be an optimum duration of compression stress, and in view of Hirsch's observation on elasticity this seems likely to be of the order of one minute.

Intra-articular fibrocartilages may be deformed in a variety of ways by different types of stress, but in relation to the present problem only two of these stresses are of importance. Thus fibrocartilages of annular form may experience a circumferential tensile stress. Fairbank (1948) and Mathur, McDonald & Ghormley (1949) have demonstrated that stress of this kind causes an appreciable deformation, and in my own experience this deformation is elastic in nature. On the other hand, all intra-articular fibro-cartilages may be subjected to compression stress by the neighbouring bone ends and my own observations have shown that they react to such stress in a manner similar to articular cartilage.

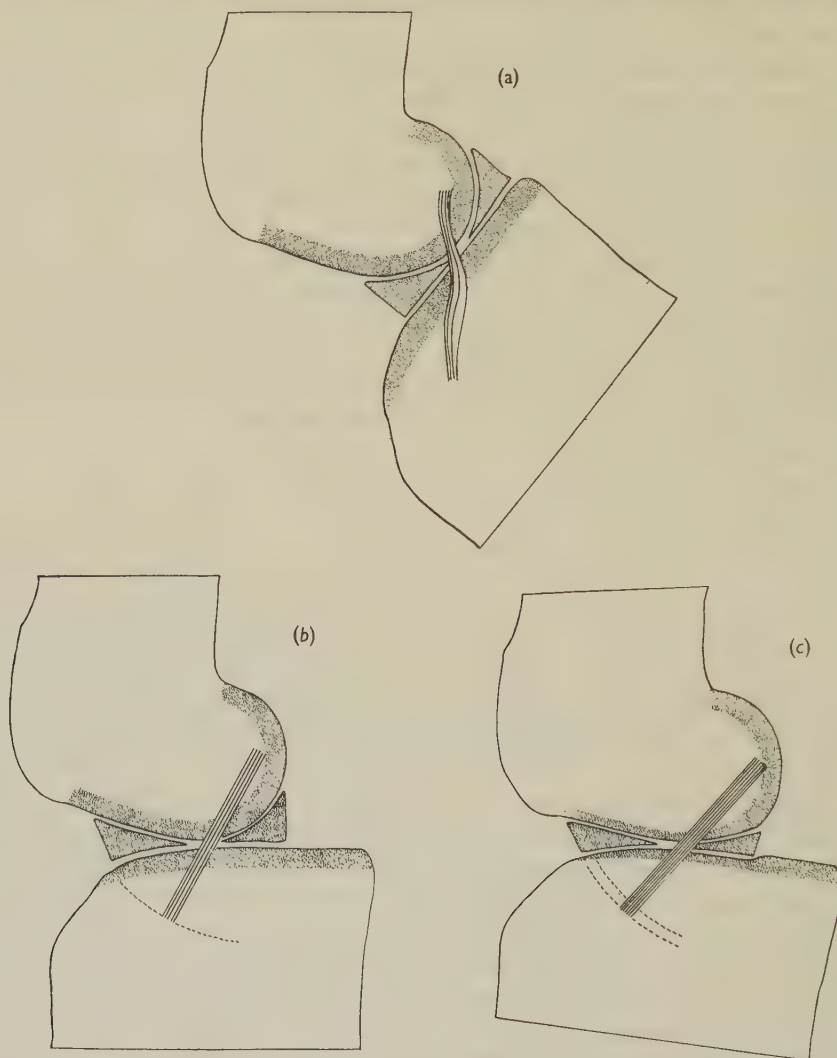
The elastic deformation caused in joint ligaments by tensile stress was recognized by Annovazzi (1928), and some features of this property have recently been described (Smith, 1954*b*). It has to be recognized in the present context that the temporary elongation produced in a joint ligament by a large load of short duration is no infinitesimal change requiring special methods for its demonstration. On the contrary, it is a gross elongation of up to 20 % of the resting length, and it can be readily appreciated with the unaided eye.

Furthermore, the stress/strain relationship gives no support to the view that elongation of a ligament is dependent on stretching of so-called elastic fibres and straightening of sinuous collagen bundles: rather it corroborates that it is dependent on elongation of the collagen fibres which are themselves deformable and elastic.

On the basis of the tissue properties described above it is postulated that the articular mechanism at the knee joint operates in the manner illustrated by the three diagrams in Text-fig. 2. These illustrations represent the knee joint in three different positions: they are essentially schematic and some features have been intentionally exaggerated. In each diagram the femur above articulates with the tibia and a meniscus below. The opposed femoral and tibio-meniscal surfaces are spirally curved, the curvature becoming progressively more acute from before backwards. The deformable tissues deep to these surfaces are indicated as stippled areas. The single joint ligament is so orientated as to tighten during extension. It is generally accepted that when the joint is extended from a position such as that in Text-fig. 2*a*, neither the articular surfaces nor the joint ligaments offer any resistance to the movement. Thus Haines (1941) has pointed out that, although both cruciate ligaments are then taut, the tension in one is in equilibrium with that in the other, and Barnett (1953) has demonstrated that although the lateral tibio-meniscal surface may be congruent with its femoral counterpart well before full extension is reached, there is no locking, because its curvature in the sagittal plane is subsequently rendered less acute by forward movement of the meniscus on the tibia (Text-fig. 2*b*). At or about the position indicated in Text-fig. 2*b* the curvature of the lateral tibio-meniscal surface becomes maximal, the paired femoral and tibio-meniscal surfaces become congruent, and the ligamentous apparatus of the joint becomes taut, but the advent of these factors does not necessarily limit extension. On the contrary, in proportion to the magnitude of the extending force, the joint ligaments will be stretched and the articular surfaces will be moulded to a new form, so that the joint will move, say,

to the position indicated in Text-fig. 2*c* before the stresses in the deformed tissues are sufficient to arrest movement.

Thus it is postulated that the exact limit of extension at the knee joint varies with the magnitude of the extending force. Furthermore, it is suggested that in relation



Text-fig. 2. Diagrammatic representation of movement at the knee joint. The interrupted lines in (b) and (c) indicate the elongation of the ligament which occurs during movement between these two positions.

to all but the smallest extending forces, the terminal degrees of joint movement are only permitted by tissue distortion, and are therefore resisted in ever increasing measure by the distortion stresses in those tissues. This hypothesis is in effect a compromise between the view of Strasser (1914) and Fick (1911) who believed that the deformability of articular cartilage was such as to prevent any effective locking

of joint surfaces, and the more recent concept of Walmsley (1928) who held that any flexibility in the locking mechanism was negligible. It receives some support from the observations which MacConaill (1945) has made on the function of the talo-calcaneo-navicular joint, and it is in keeping with certain observations of Brantigan & Voshell (1941), who noted that the position of full extension at the knee joint varied with the extending force, and that the menisci were compressed—that is the tibio-meniscal surfaces were remoulded—in the 'hyperextended' position.

The nature of the extra-articular resistance

It is considered that the extra-articular tissues which, by their resistance to stretch, exert an appreciable passive resistance to extension at the knee joint, are the flexor muscles, and the deep fascia and skin over the popliteal fossa.

It has long been recognized that an inactive or denervated muscle is elongated by load; that the extent of the elongation is related to the duration of the load; and that, within certain limits, the muscle recovers its original length when the load is removed (Langelaan, 1915). It is now considered (Fulton, 1949) that this elasticity is referable, not to the contractile actomyosin, but to the sarcolemma and fibrous framework of the muscle. As joint movement separates the attachments of an inactive muscle, the muscle becomes taut before its resting length is reached, and thereafter the tension—and consequently the resistance to the movement—increases at an increasing rate until the joint movement is complete (Fulton, 1949). The joint movement may of course stimulate contraction of the muscle through the myotatic reflex, but even if this occurs the passive tension is added to, and may be greater than, the active tension (Creed, Denny-Brown, Liddell & Sherrington, 1932). Thus in extension at the knee joint a terminal range of the movement is presumably resisted by passive tension in the flexor group of muscles.

The functions which are generally attributed to the deep fascia of the limbs are those of protection, the maintenance of shape, the promotion of venous return and the provision of extra-osseous muscle origins, but the additional function of acting as a remote ligament to the joints which it crosses is usually recognized only in the plantar aponeurosis. It is considered, however, that this ligamentous function is attributable to many other areas of fascia, and in particular to that covering the popliteal fossa. This fascia is attached proximally to the iliac crest and distally to the medial tibial condyle, the head of the fibula, the malleoli and the calcaneum. When the knee joint is fully extended a tension can be palpated in the tissues covering the central part of the popliteal fossa—a tension which can be accentuated by dorsiflexing the ankle or flexing the hip. It is considered that this tension is referable in large measure to the deep fascia, but the skin and the popliteal neurovascular bundle must also be regarded as minor contributory factors. Gratz (1931) showed that deep fascia was readily and proportionately elongated by stress and that it was, moreover, highly elastic, returning to its original dimensions when relieved of stress. It is probable therefore that the deep fascia becomes taut before full extension of the knee joint is reached, and resists movement in steadily increasing measure over the subsequent terminal range.

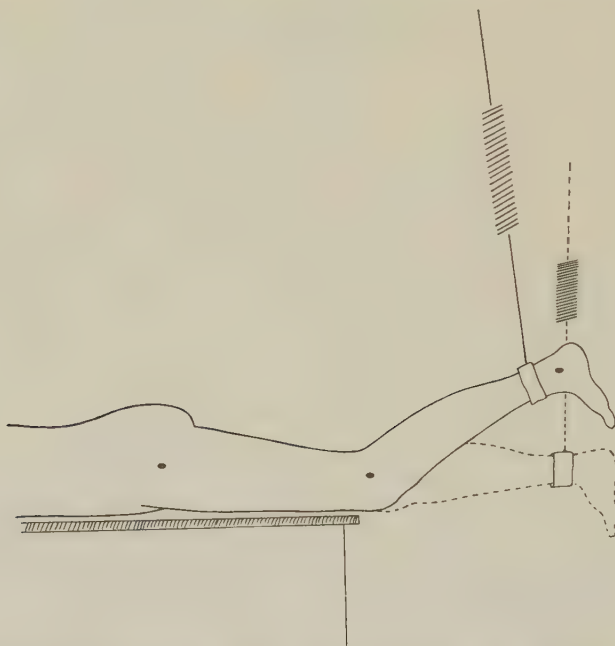
It is a matter of common experience that skin is stretched by tensile stress and returns elastically to its original dimensions when relieved. The simple manœuvre

of pinching up the skin over the popliteal fossa shows that the skin tension increases progressively over a wide terminal range of extension, and it follows that this tension exerts some restraint on the movement.

It is considered therefore that a terminal range of extension at the knee joint is resisted collectively by passive tension in the flexor muscles and in the relevant areas of skin and fascia.

FINDINGS

The nature of the articular and extra-articular resistance to extension at the knee joint and of their sum—the total passive resistance—to extension can be substantiated and placed on a quantitative basis by the following experiments.



Text-fig. 3. Determination of the total passive resistance to extension at the knee joint. The leg is shown in two of the positions which it occupies during the experiment.

Experimental assessment of the total passive resistance to extension

The experiment illustrated in Text-fig. 3 was performed on the knee joints of three young adult subjects. In each case the experiment was carried out while the subject was anaesthetized and under the influence of a relaxant before an operation for a mild chronic condition. Dr Shearer, to whom acknowledgements are made later in this paper, informs me that anaesthesia was induced by 3.75 g. thiopentone. An intravenous injection of 5.0 mg. decamethonium iodide (Eulissin) was then given and anaesthesia was continued by rhythmic inflation with gas and oxygen (2:1) with carbon dioxide absorption. The patient was completely apnoeic and all muscles were completely flaccid on manipulation. On this evidence, together with the dose

of relaxant administered, it was assumed that all limb muscles were completely relaxed.

In each experiment black markers were placed over the tip of the lateral malleolus, the lateral femoral epicondyle and the tip of the greater trochanter. The first two were regarded as marking the axes of the knee and ankle joints. The relationship of the third marker to the centre of the head of the femur is variable: in forty-two femora examined the distance of the centre of the femoral head in front of the greater trochanter varied from zero to 1 in. with an average of 0.6 in. This average figure has been used in the three subjects examined. It is appreciated that its use involves an approximation but the maximum resulting error in absolute measurements of the angulation of the knee joint is only about 2° .

In each experiment the subject was placed in the prone position so that the knee joints lay just beyond the edge of the trolley. The leg to be examined lay passively in a sling which was connected above to a calibrated tensile spring. A loop was attached to the upper end of the spring and by this means an assistant could raise or lower the leg at will. Commencing with an angle of about 140° between the leg and thigh, the leg was gradually lowered until extension was naturally limited. While the movement was in progress the relative positions of the leg and thigh and the length of the spring were recorded on cine film at sixteen frames per second. Subsequently an enlarged tracing was made of every fourth frame, and from these tracings the several quantities detailed below were measured.

The experiment was then repeated with the subject in the supine instead of the prone position, the knee joint being raised in stages in this instance between about 140° and 175° .

The forces operating at the knee joint during these experiments are shown in Text-fig. 4. B is the angle between the leg and thigh and A the angle between the leg and the horizontal. S is the tension of the tensile spring in pounds and L the minimal distance in feet from the line of the spring to the knee axis. W is the weight of the leg and foot in pounds and D the distance in feet of the centre of gravity of these parts from the knee axis. R is the total passive resistance to extension and is regarded arbitrarily as a torque acting at a distance of one foot from the knee.

In the first experiment (Text-fig. 4a) the torque resisting extension when the angulation of the knee joint is B° is

$$R_B = WD \cos A_1 - S_1 L_1. \quad (1)$$

The resistance at the same angulation in the second experiment (Text-fig. 4b) is

$$R_B = S_2 L_2 - WD \cos A_2. \quad (2)$$

In both equations the quantities W and D are unknown, and neither can be directly measured. However, the value of the factor (WD) can be calculated because the passive resistance to extension must be the same in the two experiments for equal angulations of the knee joint. In other words, for any value of B

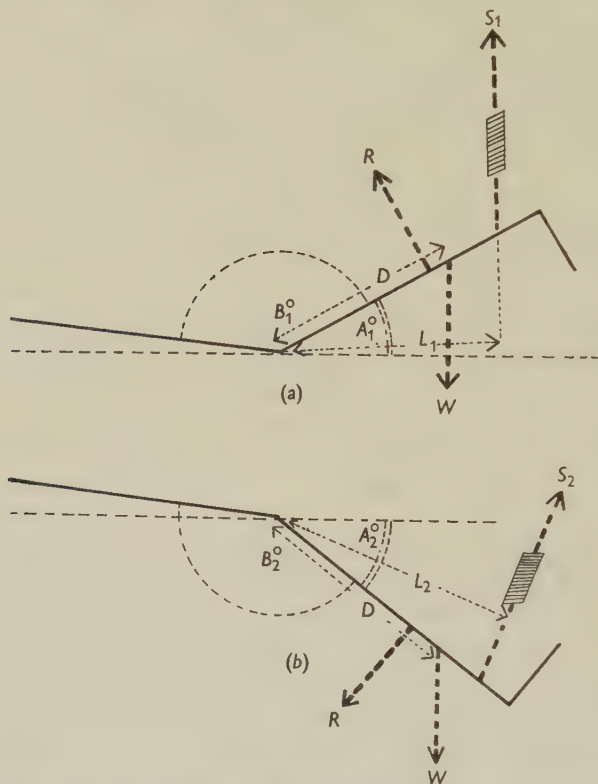
$$WD \cos A_1 - S_1 L_1 = S_2 L_2 - WD \cos A_2,$$

$$\text{or} \quad WD = \frac{S_2 L_2 + S_1 L_1}{\cos A_1 + \cos A_2}. \quad (3)$$

Once the value of (WD) is known R can be calculated for all values of B from equation (1).

Thus in the first subject the values of A_1 , A_2 , (S_1L_1) and (S_2L_2) for different values of B were as shown in Text-fig. 5.

Substituting these values in equation (3) the values of WD were calculated for values of B between 145° and 175° , and it was found to vary between 6.9 and 7.1 with an average of 7.0 lb. ft. It is considered that this method of assessing the value



Text-fig. 4. The forces operating during the experiments described in the text, (a) with the subject prone, and (b) with the subject supine. In each case the solid lines indicate the positions of the thigh, leg and foot.

of WD is more accurate than the use of the formulae of Braune & Fischer, quoted by Fenn (1930a), which are dependent on observations on the cadaver and necessarily take no account of individual variations.

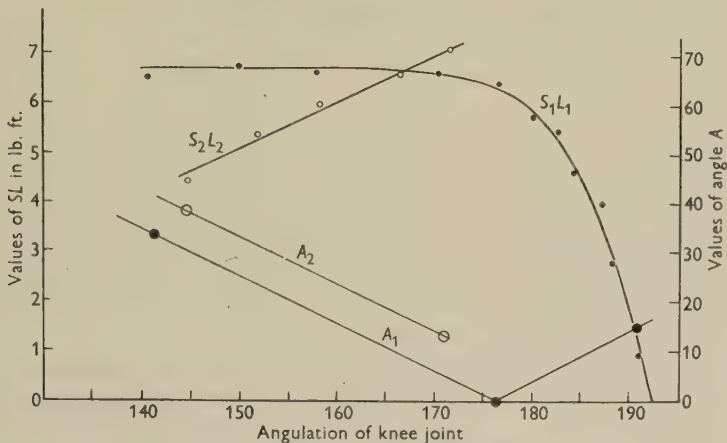
Using this value of WD and the values of A_1 and S_1L_1 in Text-fig 5, the total passive resistance to extension (R) at different angulations of the knee joint (B) can be calculated from equation (1).

The values of the total passive resistance to extension between 140° and full extension in the three subjects examined are shown in Text-fig. 6a. The results in the three individuals are closely similar, and their average is shown in Text-fig. 6b. It is apparent that at 150° the resistance is a negative quantity, that is, the stresses in the tissues tend to *promote* extension and resist flexion. At about 160° the tissue

stresses are in equilibrium, promoting neither flexion nor extension. Thereafter, the passive resistance to extension increases until, when the leg and thigh are in alignment, it has a value of 1.4 lb. ft. Beyond this angulation the resistance increases at a progressively increasing rate and at 192° , the position at which extension was naturally limited, it amounts to 7.0 lb. ft.

Experimental assessment of the articular resistance to extension

The experiment was carried out on four lower limbs which had been amputated through the mid-thigh not more than $1\frac{1}{2}$ hr. earlier. The skin and fascia around the knee joint, and all the muscles directly associated with the joint, were divided at convenient levels but not removed. The leg was then divided at approximately the junction of the upper and middle thirds. Thereafter steel pins were driven into

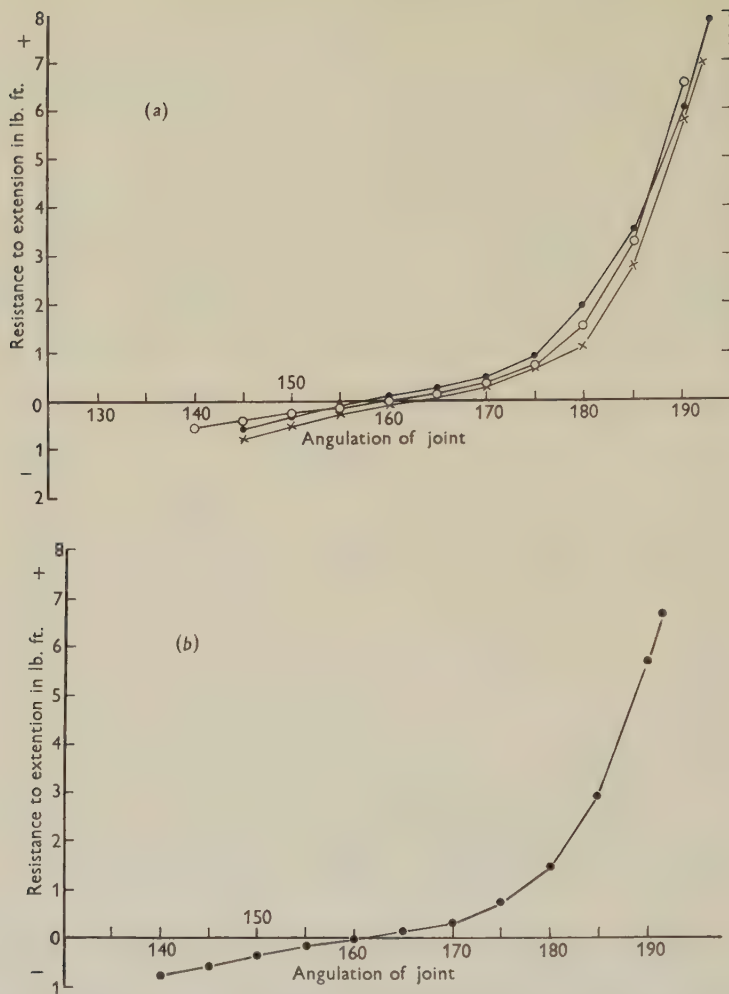


Text-fig. 5. Graphs showing the values of A_1 , A_2 , (S_1L_1) and (S_2L_2) for different values of B in the first subject.

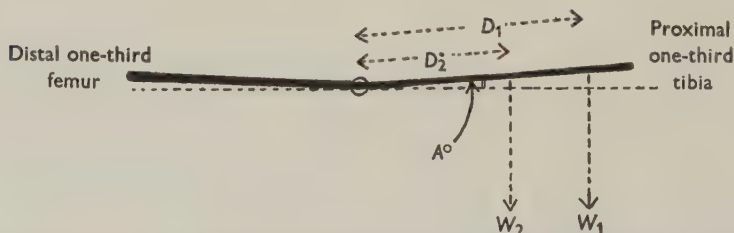
the posterior aspects of the femur and the tibia close to the joint and in a sagittal plane, so that any change from the initial angulation of the knee joint could be recorded by photographing the limb from the side. The femur was fixed with its anterior aspect facing downwards, and after the knee joint had settled into extension the limb was photographed (Pl. 1, fig. 7). Subsequently weights were applied, in increments of 2 lb. to a maximum of 20 lb., to the stump of the leg at a measured distance from the axis of the knee joint (0.48 ft.) and after each addition the limb was re-photographed and the relative angulation of the knee joint measured. The upper third of the leg was then detached from the femur and its weight (2.6 lb.) and the distance of its centre of gravity from the knee axis (0.35 ft.) determined: the same factors were then determined for the segment consisting of the lower two-thirds of the leg and the foot (3.8 lb. and 1.24 ft.). The figures given in brackets apply to one of the four specimens examined.

As indicated in Text-fig. 7, the total extending torque operating on the knee joint at any stage of the experiment is

$$\text{torque} = \cos A(W_1D_1 + W_2D_2) \text{ lb. ft.} \quad (4)$$



Text-fig. 6. The total passive resistance to extension at the knee joint (a) in the three subjects examined, (b) the average value.



Text-fig. 7. The lower part of the femur is indicated by the thick line on the left of the diagram and the upper third of the leg by the thick line on the right. The circle shows the position of the knee axis.

W_2 is the weight of the upper third of the leg in lb. acting downwards from the centre of gravity of that part, which is situated D_2 ft. from the knee axis. W_1 is the applied load acting at a distance of D_1 ft. from the same axis. A is the angle between the leg and the horizontal. Throughout these experiments A was small (less than 8°), and therefore $\cos A$ was almost unity; furthermore, in the specific experiment being described $W_2 D_2 = 0.91$ and $D_1 = 0.48$, and therefore equation (4) becomes

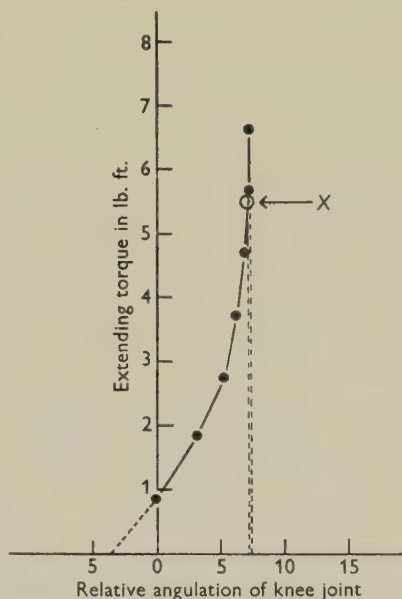
$$\text{torque} = 0.91 + 0.48 W_1 \text{ lb. ft.}$$

In Text-fig. 8 the total extending torque acting at the knee joint is plotted against the relative angulation of the joint. The point X on this graph represents the position in which the limb would have settled if the lower two-thirds of the leg and the foot had not been detached and no loads had been applied, for in those circumstances the total extending torque would have been

$$\begin{aligned} \text{torque} &= 0.91 + (3.8) (1.24) \\ &= 5.62 \text{ lb. ft.} \end{aligned}$$

In each stage of the experiment described above the extending torque acting at the knee joint is equalized by the passive resistance of the articular mechanism, so that the graph in Text-fig. 8 indicates the magnitude of this passive resistance over the terminal range of extension.

Thus it is apparent that the function of the articular mechanism at the knee joint is not confined to one position of the joint. On the contrary, the movement of extension is resisted by the mechanism over a range of about 10° , and may be limited by it anywhere in that range depending on the magnitude of the extending torque. When the knee joint is extended by the weight of the leg and foot it is practically at the limit of its range: increase in the extending torque does produce some additional extension, but this is very slight. The specimens in Pl. 1, figs. 6 and 7, illustrate this property of the articular mechanism.



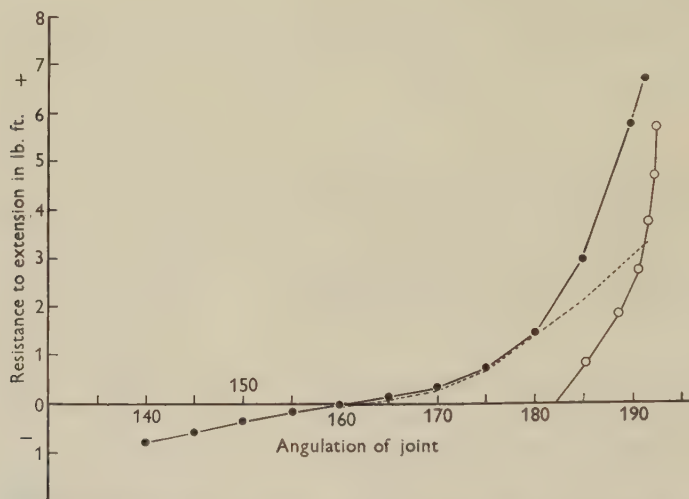
Text-fig. 8. The relationship between the extending torque and the angulation, in a stripped preparation of the knee joint.

Assessment of the extra-articular resistance to extension

I have been unable to find any method of measuring the extra-articular resistance directly. In the living subject it is impossible to eliminate the articular mechanism, and in amputated lower limbs—apart from the rare hindquarter amputation—many of the extra-articular tissues which would normally resist extension have no proximal attachment. However, despite this difficulty, an indirect assessment can be reached owing to the fact that the resistance exerted by the extra-articular tissues alone, is equal to the difference between the total passive resistance (Text-fig. 6*b*) and the resistance of the articular mechanism (Text-fig. 8). Unfortunately, the graphs in

these two figures cannot be exactly correlated; they refer to the knee joints of different individuals of different ages, and in the experiment expressed in Text-fig. 8, the exact angulations of the knee joint were unknown because the specimen necessarily lacked the upper half of the femur. Nevertheless, it is considered that the tentative correlation made in Text-fig. 9 is sufficiently exact for it to be said that from about 160° to about 180° passive resistance to extension is due entirely to the extra-articular tissues, and that thereafter the movement is resisted by the articular and extra-articular tissues together.

Furthermore, it indicates that over the greater part of the range in which the extra-articular and articular mechanisms operate together, the former is the greater of the two factors.



Text-fig. 9. The passive resistance to extension at the knee joint. The total passive resistance is represented by the upper solid line, the extra-articular resistance by the interrupted line, and the articular resistance by the lower solid line.

It is apparent from these observations that whenever extension at the knee joint is arrested, or prevented, at an angulation of more than about 160° , this is achieved, in part at least, by the passive resistance which is automatically exerted by the tissues of the part. The movement may be limited by the passive resistance alone, or by the passive resistance operating in conjunction with active contraction of the flexor muscles, and the only factors which influence the choice of mechanism are the magnitude of the extending torque which has to be resisted, and the angulation at which the knee joint must be stabilized.

The total passive resistance to extension, and the articular and extra-articular resistances which are illustrated in Text-fig. 9, are those operating in one particular set of circumstances, namely when the hip joint is practically in full extension and the foot is at right angles to the leg. It will be apparent that flexion at the hip or dorsiflexion at the ankle, although having no effect on the articular mechanism at the knee joint, must tighten the tissues involved in the extra-articular mechanism

and thus increase the range and magnitude of extra-articular resistance. But despite this variation in the resistance to extension at the knee joint with variations in the positions of the hip and ankle joints, it is considered that the positions of these joints in standing make the resistance illustrated in Text-fig. 9 approximately applicable to the study of the stability of the knee joint during that act.

It is also to be noted that the resistance of the articular limiting mechanism illustrated in Text-fig. 8 is that exerted in the non-weight bearing limb. It is apparent that weight bearing will *per se* cause compression of the tissues deep to the articular surface, and that if it were possible to measure the resistance of the articular mechanism alone in the living subject after prolonged weight bearing, it would be rather less and would operate over a slightly smaller range.

There is another aspect of the passive limiting mechanism which is of more academic interest. It has already been observed that in relation to two of the tissues involved in the mechanism, namely cartilage and muscle, the relationship between a given stress and the resulting deformation is not constant but that, within certain limits, it varies with the duration of the stress. For this reason it is to be presumed that when a prolonged extending torque of constant magnitude is passively limited, the limitation will not be absolute. On the contrary, it is to be expected that, as long as the extending torque continues to act, extension at the knee joint will proceed at a slow and gradually decreasing rate until equilibrium is finally established. This phenomenon can be observed in the living subject. When a subject lies passively in the supine position with no support between the sacrum and the lower third of the leg (Text-fig. 10), it is found that after 10 min. the anterior surface of the patella has descended half a centimetre, a displacement which represents an increase in extension of about 1.7° . In other words, prolonged stress acting on the knee joint creates a new limit of extension, and the normal limit, if such a term may be applied to it, is re-established only after a period of rest.

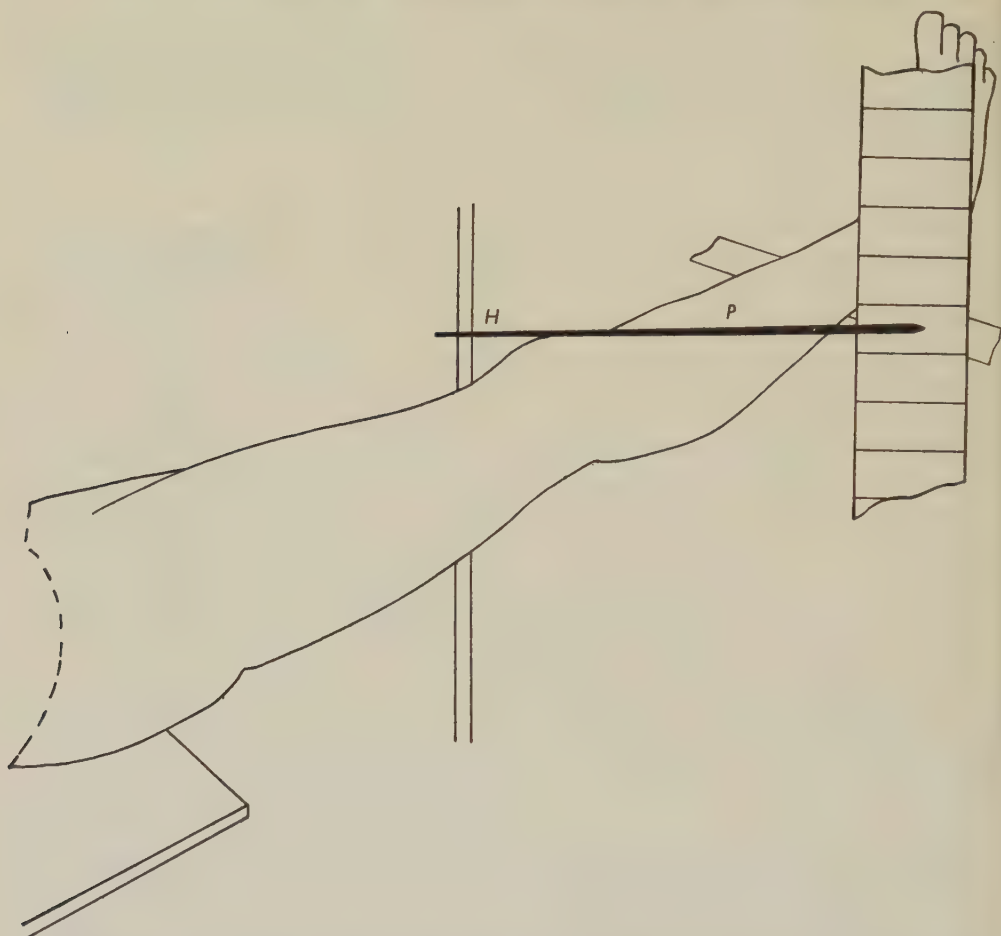
The influence exerted by the passive limiting mechanism on the knee joint in a number of common circumstances is discussed below.

THE STABILITY OF THE KNEE JOINT IN SYMMETRICAL STANDING

In the symmetrical form of standing (Smith, 1953), illustrated in Pl. 1, fig. 1, each lower limb transmits half the weight of that part of the body above the knee joints (K). The extending torque operating at each knee joint in this posture is the product of K and F , when F is the horizontal distance which separates the line of the centre of gravity of that part of the body above the knee joints from the transverse axis of the knee joint (Text-fig. 11).

Certain approximations have to be made before this extending torque can be calculated. Thus the weight of the leg and foot cannot be measured in the living subject, and in the subsequent calculations it has been regarded as being equal to the measurable quantity WD (see p. 243), because D is approximately equal to 1 ft. in most subjects. Secondly, the position of the centre of gravity of that part of the body above the knee joints cannot be determined in the standing subject, and it has consequently been assumed to lie in the same coronal plane as the centre of gravity of the whole body.

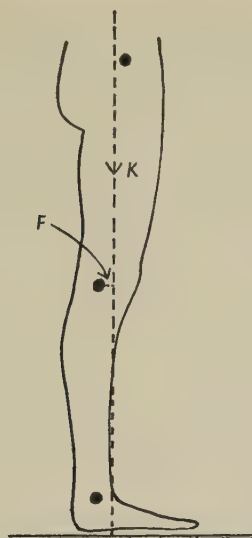
Thus far in this paper the transverse axis of the knee joint has been consistently identified with the lateral femoral epicondyle because a slight error in its position has been of little significance. In the present problem, however, the exact position of the axis must be known. Its position at various angulations of the knee joint has therefore been determined from a series of lateral photographs of the lower



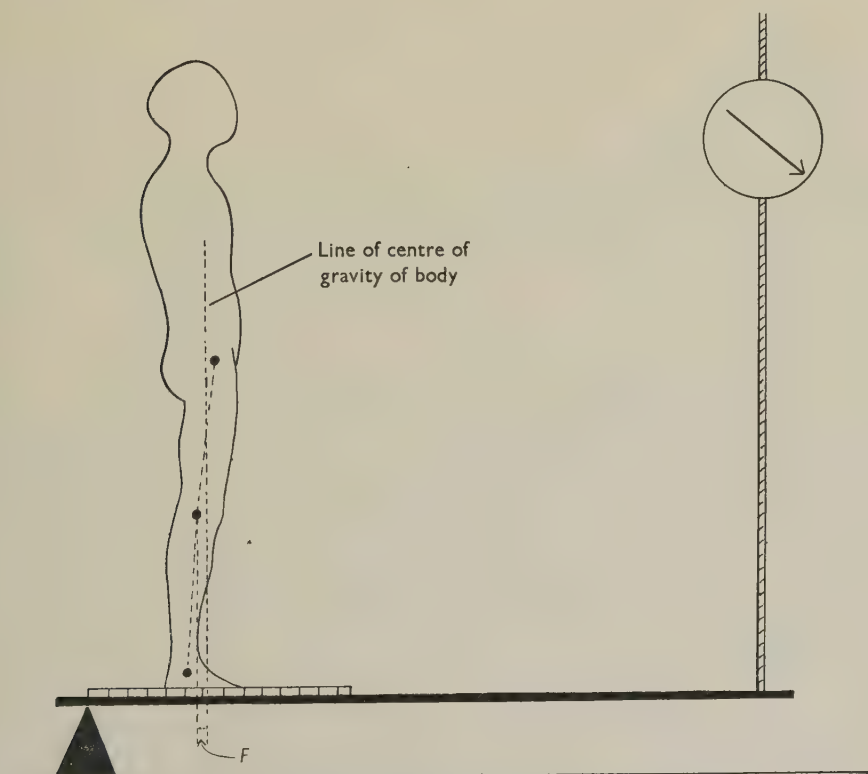
Text-fig. 10. The lower limb, supported only at the sacrum and the lower third of the leg. A pointer *P*, lying at right angles to the long axis of the limb is hinged at *H*; it rests on the anterior surface of the patella and its free end moves over a vertical scale.

limb taken at different angulations. These photographs were superimposed and the position of the knee axis constructed from the arc traversed by a marker on the lateral malleolus.

To determine the extending torque on the knee joint, black marks were placed over the greater trochanter, the lateral femoral epicondyle and the lateral malleolus, and thus prepared each subject stood symmetrically on a broad board which rested at one end on a fulcrum and was suspended at the other through a spring balance (Text-fig. 12).



Text-fig. 11. The lower limb in symmetrical standing. The black markers indicate the positions of the axes of the hip, knee and ankle joints.



Text-fig. 12. Determination of the extending torque acting at the knee joint during symmetrical standing.

An inch scale was applied to the side of the board extending from the fulcrum towards the suspension. While the subject was standing a photograph was taken from one side to include the subject, the fulcrum, the inch scale and the spring-balance dial. On this photograph, the previously determined position of the knee axis was marked so that the distance of this axis in front of the fulcrum and the angulation of the knee joint could be measured. Thereafter the line of the centre of gravity of the body was constructed from the fact that its distance in front of the fulcrum is equal to the product of the balance reading and the distance between fulcrum and suspension, divided by the body weight.

In one subject (J. S.) (Pl. 1, fig. 4) the following values were noted:

Body weight	= 161 lb.
Half the weight of that part of the body above the	
knee joints $\left(K = \frac{\text{body weight}}{2} - WD\right)$	= 72.3 lb.
Distance from fulcrum to suspension	= 5 ft.
Reading on spring balance	= 12.5 lb.
Distance of centre of gravity of body in front of fulcrum	= $\frac{5 \times 12.5}{161}$
	= 0.39 ft.
Distance of knee axis in front of fulcrum	= 0.32 ft.
Distance of centre of gravity of body in front of knee	
axis (F)	= 0.07 ft.
Extending torque on knee joint ($K \times F$)	= 5.1 lb. ft.
Angulation of joint	= 186°

Thus in this subject during symmetrical standing, the knee joint is 6° short of full extension and the extending torque which must be resisted for the knee joint to be stable amounts to 5.1 lb. ft. It is apparent that the data expressed in Text-fig. 9 cannot be exactly applied to the present subject, but nevertheless there is sufficient uniformity between different subjects for the correlation to be regarded as approximately correct. It is considered, therefore, that in the present subject during symmetrical standing the knee joint is within the sphere of influence of both the articular and the extra-articular passive limiting mechanisms. In these circumstances the articular mechanism exerts a resistance to extension of 0.9 lb. ft., the extra-articular tissues exert a passive resistance of 2.7 lb. ft., making a total passive resistance to extension of 3.6 lb. ft. Thus, what is required from the postural activity of the flexor muscles to stabilize the knee joint is a flexing torque of 1.5 lb. ft. In other words, of the total flexing torque which is necessary to counteract the force of gravity and stabilize the knee joint during symmetrical standing, about 50% is derived from the passive resistance of the extra-articular tissues, about 30% from the postural activity of the flexor muscles and about 20% from the passive resistance of the articular mechanism.

THE STABILITY OF THE KNEE JOINT IN ASYMMETRICAL STANDING

In the asymmetrical form of standing (Smith, 1953), illustrated in Pl. 1, fig. 2, one lower limb (the supporting limb) transmits the greater part of body weight, while the other (the balancing limb) acts as a prop controlling the forward sway of the

body. The weight transmitted through each limb can be measured if the subject stands asymmetrically with the feet on separate weighing machines.

In this posture the tendency of body weight is not to carry the body and thigh forwards over the knee joint as in symmetrical standing, but to force the knee joint of the supporting limb backwards in relation to both the hip and ankle joints. Thus assuming that the hip joint is stabilized and that the resistance to plantarflexion at the ankle joint is negligible, the extending torque operating at the supporting knee joint is the product of W_s and C (Text-fig. 13) when W_s is the weight transmitted through the supporting limb and C the vertical distance from the knee axis of a line joining the axes of the hip and ankle joints.

In the subject J. S. (Pl. 1, fig. 5) the weight transmitted by the supporting limb was $W_s=141$ lb. and that transmitted by the balancing limb was $W_b=20$ lb. The distance C , as measured from a photograph, was 0.11 ft. and therefore the extending torque at the knee joint was $W_s C=15.5$ lb. ft.

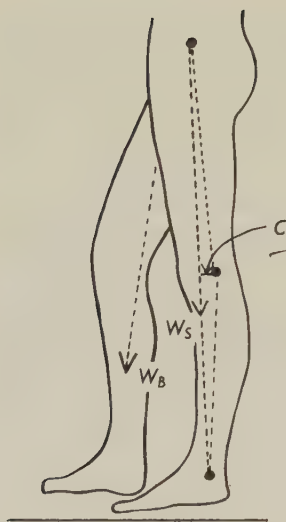
The angulation of the supporting knee joint in this posture was 190° , which as would be expected from the greater extending torque, is appreciably greater than the angulation of either knee joint in symmetrical standing. The data in Text-fig. 9 which can be approximately applied to the present subject shows that at this angulation the total passive resistance to extension is 5.7 lb. ft., of which 3.4. lb. ft. is due to the extra-articular mechanism and 2.3 lb. ft. to the articular mechanism. It is considered therefore that of the total flexing torque necessary to counteract the force of gravity and stabilize the knee joint of the supporting limb during asymmetrical standing, about 65% is derived from the postural activity of the flexor muscles, 20% from the passive resistance of the extra-articular tissues and about 15% from the resistance of the articular mechanism. Moreover, it is to be noted that although in asymmetrical standing the knee joint is only 2° short of full extension, and is thus well within the range of movement affected by the articular mechanism, that mechanism still contributes comparatively little to the stability of the knee joint.

THE ANGULATION OF THE KNEE JOINT IN WALKING AND RUNNING

The angulation of the knee joint during walking and running has been studied on cine films by Elftman (1939) and Fenn (1930*b*), and the graphs in Text-fig. 14 have been compiled from the data in their papers. The pattern of movement is similar in the two activities: peaks of extension at the beginning and end of the stance phase are separated by the maximal weight-bearing period during which the joint is rather less extended; during the swing phase the joint is flexed and the limb shortened.

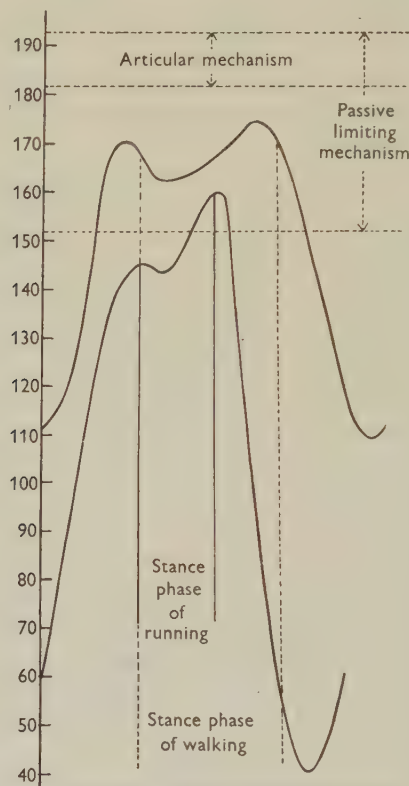
Two features are relevant to the present paper. In both walking and running the knee joint moves into the passively resisted range of extension during the stance phase, but in neither activity does it enter the zone of influence of the articular mechanism. Thus the joint cannot be regarded as being locked at any phase of either walking or running (Saunders, Inman & Eberhart, 1953). Secondly, it will be noted that in running the extension at foot contact is less than at 'toe off'. It is considered that this difference is due, in part at least, to the different position of the

hip joint on those occasions. At 'toe off' the hip joint is extended and the ankle joint plantarflexed so that, as already noted on p. 248, the passive extra-articular resistance to extension at the knee joint is minimal. At foot contact, on the other hand, the hip joint is flexed so that the passive extra-articular resistance to extension at the knee joint is augmented in its effective range and magnitude.



Text-fig. 13

Text-fig. 13. The lower limbs in asymmetrical standing. The black markers indicate the positions of the axes of the hip, knee and ankle joints. W_S represents the weight transmitted by the supporting limb and W_B that by the balancing limb.



Text-fig. 14

Text-fig. 14. Variations in the angulation of the knee joint during walking and running.

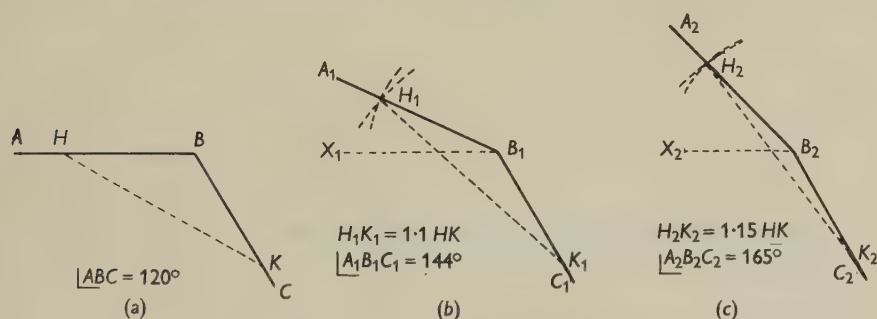
DISCUSSION

It is believed that the passive limiting mechanism which has been described in connexion with extension at the knee joint is not unique. On the contrary, because it depends on physical properties which are common to tissues in all regions of the body, it seems certain that a terminal part of every joint movement is resisted by a similar mechanism. Preliminary observations have been made on the passive mechanisms resisting dorsiflexion at the ankle joint and flexion of the vertebral column, and these taken in conjunction with the observations of Silver (1954) on the latter part have confirmed this view. However, despite the essential similarity

of these mechanisms, it is to be presumed that they will vary considerably both in the magnitude of the resistance they exert, and the range over which they operate, and certain propositions can be laid down which govern these variations. In this context the tissues involved in a passive limiting mechanism are most conveniently divided into those resisting tensile stresses, and those resisting compression stresses, and these groups will be referred to as 'tensile tissues' and 'compression tissues' respectively.

In relation to a tensile tissue, the magnitude of the resistance which it exerts to a movement will be proportional to Young's modulus and the cross-sectional area of the tissue.

The range of joint movement over which a tensile tissue is capable of exerting resistance depends on three factors. It depends first on the elastic extensibility of the tissue (E). In Text-fig. 15*a* ABC is a joint which is hinged at B and has its two arms AB and BC joined by a tensile tissue HK . If this tensile tissue is capable of an extension of 10% of its original length before rupturing, such an extension would allow the joint to move to the position shown in (*b*) of the same figure. On the other



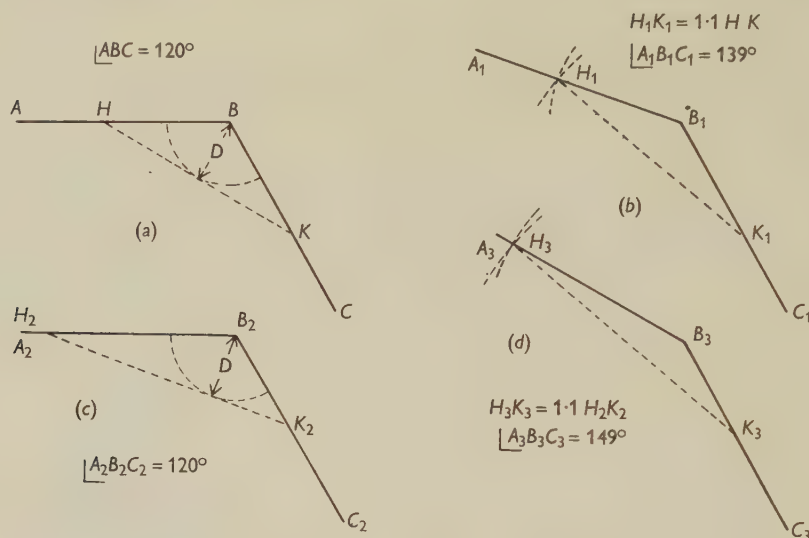
Text-fig. 15. The influence of the extensibility of a tensile tissue on the range of passively resisted joint movement.

hand, an extension of 15% would allow movement to the position shown in (*c*). It will be noted that $\angle X_2B_2A_2$ is greater than $\angle X_1B_1A_1$, that is, the range of passively resisted movement at a joint is a function of the extensibility of the related tensile tissues.

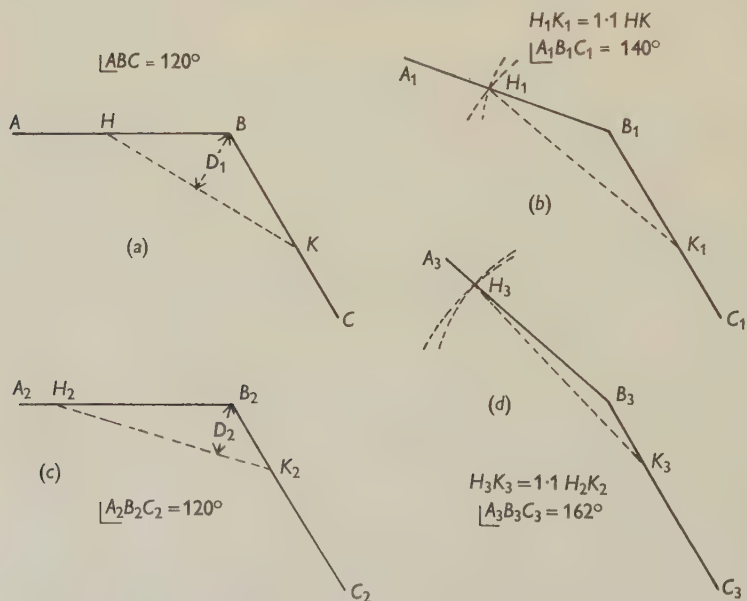
In Text-fig. 16*a* the arms of a joint ABC are joined by a tensile tissue HK , which lies at a distance D from the axis of the joint. A 10% extension of HK allows the joint to move to the position shown in (*b*), that is, it allows a passively resisted movement of 19° . In (*c*) of the same figure the arms of the same joint are linked by a tensile tissue H_2K_2 , which is longer than HK but lies at the same distance D from the joint axis. A 10% extension of H_2K_2 allows the joint to move to the position shown in (*d*), that is, it allows a passively resisted movement of 29° . Thus the range of passively resisted movement at a joint is a function of the length of the related tensile tissues.

The third factor is the distance of the tensile tissue from the axis of rotation of the joint (D). In Text-fig. 17*a* the tensile tissue HK links the arms of the joint ABC and lies at a distance D_1 from the joint axis B . A 10% extension of HK allows the joint to move to the position shown in (*b*) of the same figure, that is, it allows a

passively resisted movement of 20° . In (c) the arms of an exactly similar joint are linked by a tensile tissue H_2K_2 , which is the same length as HK but lies at a shorter distance D_2 from the joint axis. A 10% extension of H_2K_2 allows a passively resisted movement of 42° as shown in (d). Thus the range of passively resisted movement permitted by a tensile tissue is an inverse function of the distance of that tissue from the joint axis.



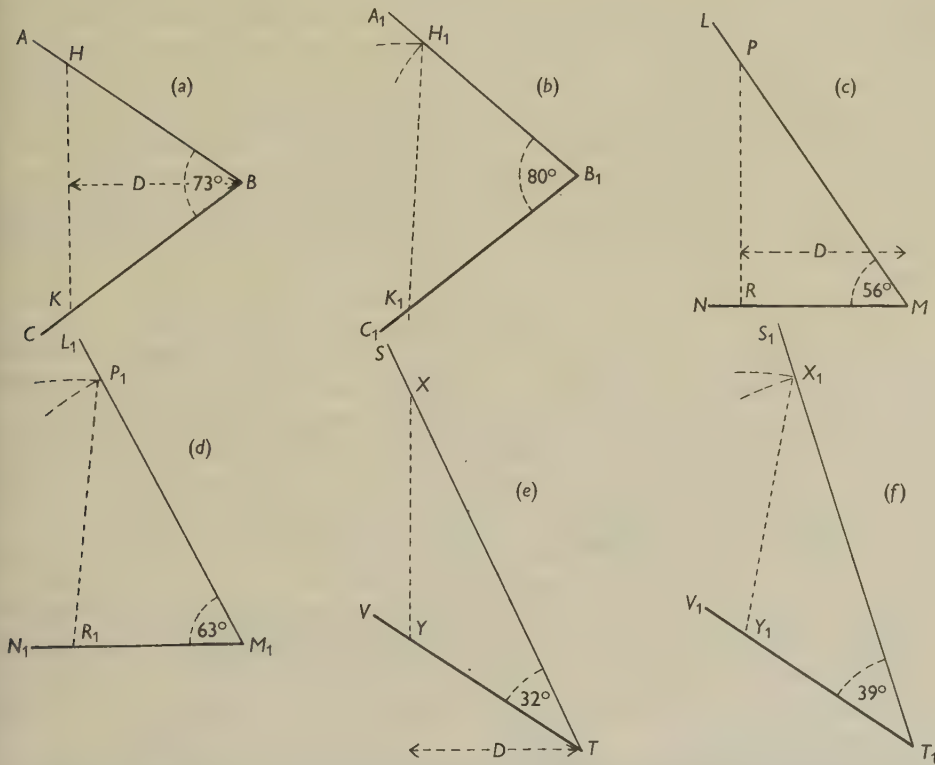
Text-fig. 16. The influence of the length of a tensile tissue on the range of passively resisted joint movement.



Text-fig. 17. The influence of the distance of a tensile tissue from the axis of rotation, on the range of passively resisted joint movement.

The range of passively resisted movement (M) which can occur at any joint is thus dependent on certain properties of the tensile tissues: it is a function of their extensibility (E), their length (L) and an inverse function of their distance from the joint axis (D). Furthermore, E , L and D are the only properties of the tensile tissue which influence the value of M .

In Text-fig. 18 *a* the joint ABC has an angulation of 73° . The arms of the joint are linked by the tensile tissue HK lying at a distance D from the joint axis. A 10% extension of HK allows a passively resisted movement of 7° (see *b*). In (*c*) of the same figure, the joint LMN has an angulation of 56° . Its arms are linked by the



Text-fig. 18. Proof that the range of movement resisted by a tensile tissue depends only on the extensibility and length of the tissue, and its distance from the axis of rotation.

tensile tissue PR , which is equal in length to HK and lies at the same distance D from the joint axis. A 10% extension of PR again permits a passively resisted movement of 7° (see *d*). Similarly, a 10% extension of the tensile tissue XY in (*e*) allows a passively resisted movement of 7° in the joint STV (*f*).

In relation to compression tissues, the range of resisted movement at any joint is a function of the deformability of the articular surfaces, and this in turn depends on two factors: first, the compressibility of the tissues deep to the articular surfaces, and secondly, the rigidity of the union between different parts of those tissues.

The compressibility of the tissues deep to a joint surface is a function of their thickness, and is greater in relation to a thick layer of articular than to a thin layer, greater at those joints possessing intra-articular fibro-cartilages than in those devoid of those structures, and greater in relation to an incompletely ossified epiphysis than to a mature bone.

When an articular surface extends over a continuous layer of articular cartilage which is supported by a single bone, that surface can be appreciably deformed only by distortion of the cartilage. But all articular surfaces do not conform to this pattern. In the child, the proximal surfaces of the shoulder and hip joints consist of areas of articular cartilage supported by a number of discrete bony elements, which are themselves united by cartilage. And in the adult, the proximal surfaces at the ankle joint and most of the costo-vertebral joints, and the distal surface at the talocalcaneo-navicular joint consist of areas of articular cartilage supported by bones which are themselves joined by ligaments. It is apparent that in such instances relative displacement of the separate supporting bones will give rise to changes in the form of the articular surface quite apart from any distortion of the compression tissues. Furthermore, as Barnett (1953) has shown at the knee joint, an intra-articular fibro-cartilage may be capable of a displacement in relation to the underlying articular cartilage, which profoundly alters the form of the total articular surface.

SUMMARY

1. A terminal range of extension at the knee joint is associated with distortion of certain articular and extra-articular tissues, and the movement is consequently passively resisted by distortion stresses in those tissues. Thus the total passive resistance to extension has been regarded as the sum of the resistances exerted by an articular and a passive extra-articular mechanism.

2. The exact nature of the articular mechanism is discussed.

3. The magnitude and the range of operation of the total passive resistance and of its articular and extra-articular parts are determined.

4. Measurements are made of the contribution of postural muscle activity and the passive resistance to extension to the stabilization of the knee joint in symmetrical and asymmetrical standing.

5. The influence of passive resistance to extension of the knee joint on walking and running is discussed.

6. The factors influencing the magnitude and range of operation of mechanisms passively resisting joint movement are discussed.

I wish to thank Prof. R. Walmsley for the help and advice which he has given me throughout this investigation. It is also a great pleasure to acknowledge the co-operation of two of my colleagues, Dr W. M. Shearer, Senior Anaesthetist in Dundee Royal Infirmary, who gave me facilities for carrying out some of the experiments, and Mr James Adamson of the Department of Natural Philosophy, St Andrews, for his advice on mechanical problems.

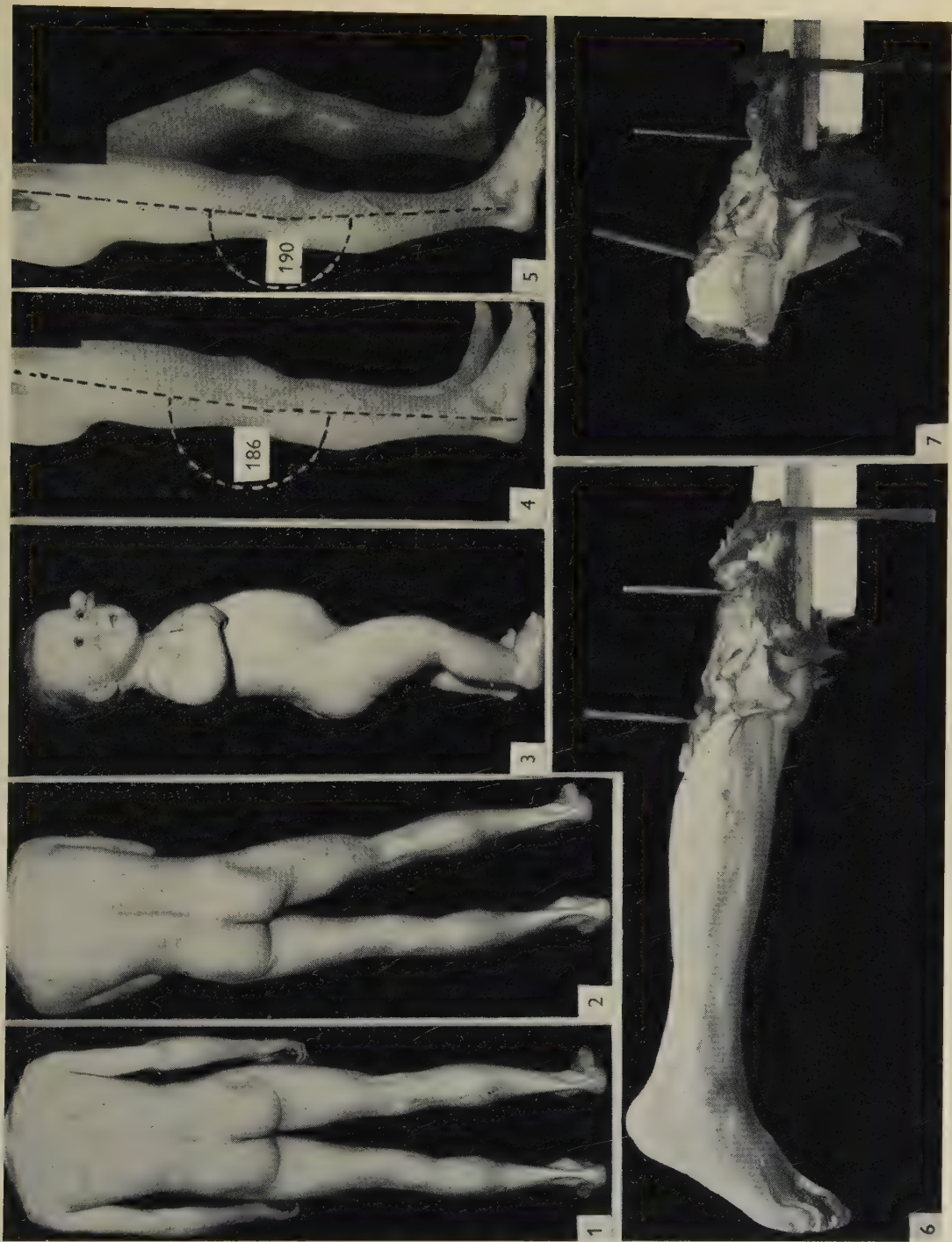
REFERENCES

- ANNOVAZZI, G. (1928). Osservazioni sulla elasticita dei legamenti. *Arch. Sci. biol., Napoli*, **11**, 467-501.
- BÄR, E. (1926). Elastizitätsprüfungen der Gelenknorpel. *Arch. Entw-Mech. Org.* **108**, 739-753.
- BARNETT, C. H. (1953). Locking at the knee joint. *J. Anat., Lond.*, **87**, 91-95.
- BRANTIGAN, O. C. & VOSHELL, A. F. (1941). The mechanics of the ligaments and menisci of the knee joint. *J. Bone Jt. Surg.* **23**, 44-66.
- BRASH, J. C. (1951). *Cunningham's Text Book of Anatomy*, 9th ed. Oxford University Press.
- BRAUNE, W. & FISCHER, O. (1891). *Die Bewegungen des Kniegelenke*. Leipzig: Hirzel.
- CLEMMESSEN, S. (1951). Some studies in muscle tone. *Proc. R. Soc. Med.* **44**, 637-646.
- CREED, R. S., DENNY-BROWN, D., LIDDELL, E. G. T. & SHERRINGTON, C. S. (1932). *Reflex Activity of the Spinal Cord*. Oxford: Clarendon Press.
- DENNY-BROWN, D. (1949). The interpretation of the electromyogram. *Arch. Neurol. Psychiat., Chicago*, **61**, 99-128.
- ELFTMAN, H. (1939). Forces and energy changes in the leg during walking. *Amer. J. Physiol.* **125**, 339-356.
- FAIRBANK, T. J. (1948). Knee joint changes after menisectomy. *J. Bone Jt. Surg.* **30B**, 664-670.
- FENN, W. O. (1930*a*). Frictional and kinetic factors in the work of sprint running. *Amer. J. Physiol.* **92**, 583-611.
- FENN, W. O. (1930*b*). Work against gravity and work due to velocity changes in running. *Amer. J. Physiol.* **93**, 433-462.
- FICK, R. (1911). *Bardeleben's Handbuch der Anatomie des Menschen*, Bd. 2, Abt. 1, Tl. 3. Jena: Fischer.
- FULTON, J. F. (1949). *A Textbook of Physiology*. Philadelphia: Saunders.
- GOODSIR, J. (1868). *The Anatomical Memoirs of John Goodsir*. Edinburgh: Black.
- GRATZ, C. M. (1931). The tensile strength and elasticity test of human fascia lata. *J. Bone Jt. Surg.* **13**, 334-340.
- HAINES, R. W. (1941). A note of the actions of the cruciate ligaments of the knee joint. *J. Anat., Lond.*, **75**, 373-375.
- HIRSCH, C. (1944). A contribution to the pathogenesis of chondromalacia of the patella. *Acta chir. scand.* **90**, (Suppl. 83), 1-106.
- HOOTON, E. A. (1947). *Up from the Ape*. New York: Macmillan.
- INGELMARK, B. E. & EKHOLM, R. (1948). Study on variations in thickness of articular cartilage, in association with rest and periodic loading. *Uppsala LäkFören. Förh.* **53**, 61-74.
- INGELMARK, B. E. (1950). The nutritional supply and nutritional value of synovial fluid. *Acta orthopaed. scand.* **20**, 144-155.
- JOHNSTON, T. B. & WHILLIS, J. (1954). *Gray's Anatomy*, 31st ed. London: Longmans.
- JOSEPH, J. & NIGHTINGALE, A. (1952). Electromyography of muscles of posture: leg muscles in males. *J. Physiol.* **117**, 484-491.
- LANGELAAN, J. W. (1915). On muscle tone. *Brain*, **38**, 235-380.
- LIPPOLD, O. C. J. (1952). The relations between integrated action potentials in a human muscle and its isometric tension. *J. Physiol.* **117**, 492-499.
- MACCONAILL, M. A. (1945). The postural mechanism of the human foot. *Proc. R. Irish Acad.* **50B**, 265-278.
- MATHUR, P. D., McDONALD, J. R. & GHORMLEY, R. K. (1949). A study of the tensile strength of the menisci of the knee. *J. Bone Jt. Surg.* **31A**, 650-654.
- MORTON, D. J. (1935). *The Human Foot*. New York: Colombia University Press.
- RALSTON, H. J. & LIBET, B. (1953). The question of tonus in skeletal muscle. *Amer. J. Phys. Med.* **32**, 85-92.
- SAUNDERS, J. B. DE C. M., INMAN, V. T. & EBERHART, H. D. (1953). The major determinants in normal and pathological gait. *J. Bone Jt. Surg.* **35A**, 543-558.
- SEYFFARTH, H. (1940). *The Behaviour of Motor-Units in Voluntary Contraction*. Oslo: Dybiwad.
- SEYFFARTH, H. (1941). The behaviour of motor units in healthy and paretic muscles in man. *Acta psychiat., Kbh.*, **16**, 79-109, 261-277.
- SILVER, P. H. S. (1954). Direct observation of changes in tension in the supraspinous and inter-spinous ligaments during flexion and extension of the vertebral column in Man. *J. Anat., Lond.*, **88**, 550.

- SMITH, J. W. (1953). The act of standing. *Acta orthopaed. scand.* **23**, 159-168.
- SMITH, J. W. (1954*a*). Muscular control of the arches of the foot in standing: an electromyograph assessment. *J. Anat., Lond.*, **88**, 153-163.
- SMITH, J. W. (1954*b*). The elastic properties of the anterior cruciate ligament of the rabbit. *J. Anat., Lond.*, **88**, 369-380.
- STRASSER, H. (1914). *Lehrbuch der Muskel- und Gelenkmechanik*. Berlin: Springer.
- WALMSLEY, T. (1928). The articular mechanism of the diarthroses. *J. Bone Jt. Surg.* **10**, 40-45.
- WEDDELL, G., FEINSTEIN, B. & PATTLE, R. E. (1944). Electrical activity of voluntary muscle in man under normal and pathological conditions. *Brain*, **67**, 178-257.
- WRIGHT, S. (1952). *Applied Physiology*, 9th ed. Oxford University Press.

EXPLANATION OF PLATE

- Fig. 1. Symmetrical standing in the adult.
- Fig. 2. Asymmetrical standing in the adult.
- Fig. 3. Standing in a child of 14 months.
- Fig. 4. The adult lower limb in symmetrical standing. The finger marks the tip of the greater trochanter of the femur.
- Fig. 5. The adult lower limb in asymmetrical standing. The finger marks the tip of the greater trochanter of the femur.
- Fig. 6. Stripped knee joint of amputated lower limb. The joint is here extended by the weight of the whole leg and foot.
- Fig. 7. The same knee joint as that illustrated in fig. 6. The joint is here extended by the weight of the proximal third of the leg only. The reduced extension at the joint is indicated by the altered position of the pins.



SMITH—OBSERVATIONS ON THE POSTURAL MECHANISM OF THE HUMAN KNEE JOINT

(Facing p. 260)

THE EVOLUTION OF THE MAMMALIAN EARDRUM AND TYMPANIC CAVITY

By C. C. D. SHUTE

Department of Anatomy, University of Cambridge

INTRODUCTION

Two opposite views on the origin of the mammalian middle ear have been expressed by Gaupp (1913) and Goodrich (1930). Gaupp held that the eardrum of reptiles was essentially supramandibular and that of mammals submandibular, so that the latter could not have arisen from the former, but must have evolved separately. Goodrich, on the other hand, declared that the tympanic membrane, middle ear cavity and Eustachian tube in reptiles and mammals were completely homologous with one another. Recently, as more has been discovered of the anatomy of mammal-like reptiles, it has become clear that the tympanic membrane is situated more antero-ventrally in mammals than in reptiles, but it is not immediately apparent at what stage the migration occurred. Watson (1953) emphasized how undesirable it would be to suppose that the change was 'saltatory', that is to say, that it involved some major jump or discontinuity in evolution. Nevertheless, he himself was led to the conclusion that in some of the synapsid ancestors of mammals the eardrum was completely lost. He based this view on the large size of the stapes, its extensive contact with the quadrate, and the lack of an obvious site for an external auditory meatus in certain members of the captorhinomorph-pelycosaur line. Below the skin, however, the original middle ear cavity supposedly remained in communication with the pharynx (this sometimes happens, though not usually, in those lizards in which the external auditory meatus and tympanic membrane have been lost), and contact with the surface was re-established in therapsids to form a new tympanic membrane and a new external auditory meatus.

Westoll (1943), and other workers, have not believed that synapsids lost and redeveloped their tympanic membranes. According to Romer & Price (1940), pelycosaurs could have possessed an eardrum, sunk as in many lizards in the depths of an external auditory meatus, between the quadrate in front and the depressor mandibulae muscle behind. Parrington (1946, 1955) showed that such a drum could have been present in cynodonts and gorgonopsids.

The problem is to explain how an eardrum originally in the reptilian position could come to be embraced by the mammalian ectotympanic bone. Gaupp (1911*b*) originally homologized the ectotympanic of mammals with the reptilian angulare, and Palmer (1913) noted the resemblance between the ectotympanic of the marsupial *Perameles* and the reflected lamina of the angulare in late cynodonts. Consequent upon their observations, it has been generally agreed that at some stage during synapsid evolution the periphery of the tympanic membrane must have reached the angulare. In spite of this, Romer & Price have demonstrated that the reflected lamina could not always have supported an eardrum. Their arguments were that

migration of the drum forwards from the reptilian site would have been prevented by the depressor mandibulae, that the stapes could not have been connected to a drum in this position, that there would often not have been room for a tympanic cavity deep to the drum, that it would have been obstructed by pterygoid muscles, and that a tympanic membrane so far forward would have interfered with movements of the lower jaw.

Some of these difficulties were met, and the awkward gap between synapsids and mammals bridged, by a theory of the evolution of the middle ear devised by Westoll (1943). Sushkin (1927) had already concluded that part of the *cavum tympani* in mammal-like reptiles generally must have approached the surface below the jaw articulation, to form a drum in the notch of the *angulare* which, he believed, was represented to some extent even in early forms. Westoll postulated that a *diverticulum*, 'either originally separate from the Eustachian tube and tympanic cavity, or much more probably an extension of the tympanic cavity itself', grew out to meet the *angulare* and eventually became supported by it. A similar suggestion had been made by Watson (1951) in his original Silliman lectures. Westoll gave this *diverticulum* the name '*recessus mandibularis tympanicus*', and illustrated its position in a *pelecosaur*, a *cynodont* and a *therocephalian*. He thought that its original function might have been to act as a resonator comparable to the vocal sacs of frogs, and that eventually its outer wall approached the skin surface around the retro-articular process of the *articulare*, which then became transformed into the *manubrium mallei* of a true eardrum of mammalian type. Later (1945), Westoll clinched his theory by homologizing Shrapnell's membrane, that is, the *pars flaccida* of the mammalian drum, with the original tympanic membrane of the ancestors of mammals, and with the eardrum possessed by modern reptiles. He interpreted the malleolar folds between the *pars flaccida* and the *pars tensa* as 'the reduced and compressed equivalent of the tissues which laterally separated the upper part of the tympanic cavity of therapsids from the *recessus mandibularis*'.

Westoll's interpretation is a neat one, and has received very general acceptance, notably from Parrington (1949) and Gregory (1951). Parrington later (1955) expressed some doubts whether a *diverticulum* of the tympanic cavity could act as a resonator. Vaughn (1955) also accepted most of Westoll's conclusions, but pointed out that his theory did not explain the course of the *chorda tympani*, which runs dorsal to the tympanic membrane of reptiles and ventral to Shrapnell's membrane in mammals. Vaughn attempted to resolve this difficulty by reference to skull morphology. He supposed that, as a result of changes in the proportions of the temporal region occurring in the early stages of synapsid evolution, the middle ear and its contents were 'driven ventrally', and 'drove the *chorda tympani* before them'. Later the *recessus mandibularis* pushed out ventrally, and the *chorda tympani* was trapped between it and the original tympanic cavity.

The purpose of the present paper is to draw attention to certain problems which the hypothesis of a *recessus mandibularis tympanicus* does not solve, and to put forward as an alternative a theory, supported by some evidence, which is in line with Westoll's other suggestion of a pharyngeal *diverticulum* distinct from the Eustachian tube.

PRESENT THEORY

A strong argument, based on morphological considerations, against the view that the mammalian middle ear has evolved from a diverticulum of the original tympanic cavity, is that it does not account for an apparent antero-ventral shift in mammals, not only of the tympanic membrane but also of the opening of the Eustachian tube. Evidence of this change in position is as follows. First, the tubotympanic recess in mammals approaches the auditory ossicles from in front, with, above it, the internal carotid artery *leaving* the region of the middle ear. In reptiles such as lizards, on the other hand, the Eustachian tube runs laterally from the pharynx, and the internal carotid *approaches* the tympanic cavity in its roof (Figs. 2, 3B, 5A). Secondly, the fenestra rotunda, bearing the secondary tympanic membrane, overlies the Eustachian tube in lizards, whereas in mammals it is in the roof of the extreme posterior cul-de-sac of the tympanic cavity, as far away as could be from the Eustachian tube. Thirdly, it is generally agreed that the tensor tympani of mammals is a transformed muscle of mastication, derived from the reptilian adductor mandibulae internus (= anterior pterygoid, pterygo-mandibularis). In reptiles the adductor mandibulae internus is ventral to the Eustachian tube (Fig. 3), and Westoll (1943) accordingly showed it in this position in his figure of the recessus mandibularis in a cynodont. In mammals, however, the tensor tympani runs in the upper part of the tympanic cavity (Fig. 5A), and its origin is dorsal to the Eustachian tube. The tensor palati muscle presents similar difficulties.

Parrington & Westoll (1941) have shown that during synapsid evolution the anterior part of the basis cranii has undergone shortening. It is doubtful whether this can account for the changed relations of the Eustachian tube, since the latter opens behind the basipterygoid process, while the shortening apparently occurs in front of it. It seems, therefore, that the concept of a tympanic diverticulum may explain some, but cannot explain all, of the features of the mammalian middle ear. Furthermore, it does not receive direct support from embryology. Ontogeny, of course, is not obliged to repeat the stages of phylogeny, but an *ad hoc* postulate of this sort would gain in plausibility if some indication of its occurrence could be seen during embryonic development. One might, for instance, expect to find in mammalian development the tubotympanic recess first growing out around the primordium of the stapes, since it is the oldest ear ossicle; and later a pouch being thrown out towards the ectotympanic which would envelop the handle of the malleus. In fact, however, no such pouch is formed, and the tubotympanic recess meets the manubrium mallei first of all (Fig. 1), reaching the stapes and the attic region of the middle ear only at a relatively late stage (Fig. 5A). This is a constant finding in eutherian mammals, and is in accordance with McClain's observations (1939) in the marsupial *Didelphys*. Similarly, unless there has been a reversal in the order of events during ontogeny, Shrapnell's membrane as an ancestral reptilian heritage should make an early appearance. Actually, it seems to be the very last part of the eardrum to develop, not appearing in man until the final month of foetal life (Keibal & Mall, 1912).

Further objections to the theory that the tympanic cavity acquired a mandibular recess during phylogeny are the following. First, it is not clear what would have

been the original purpose of such a recess. Why should a diverticulum have grown out from the tympanic cavity in the first place, meeting the angulare so conveniently for the subsequent formation of a pars tensa of the tympanic membrane? Although vocal resonators are common amongst modern tetrapods, there is no instance of one having been developed from the middle ear. The vocal sacs of frogs are not of tympanic origin, but are formed as diverticula of the buccal cavity. Secondly, it seems curious that a simple outpouching of the tympanic cavity should exert such forces during morphogenesis as to entrap the chorda tympani and compress the tissue of the malleolar folds. Thirdly, an extension of the middle ear attached to the lower jaw, even before the development of a tympanic membrane, would probably have been something of an embarrassment both to mastication and to hearing.

In the light of these difficulties, it was felt that an alternative explanation must be sought to interpret conditions in mammal-like reptiles and to account for the



Fig. 1. *Elephantulus* embryo, 14 mm. Section showing the typical mammalian relation of the tubotympanic recess to the manubrium mallei.

differences between the Eustachian tube, middle ear cavity and tympanic membrane of modern reptiles and mammals. The middle ear and pharyngeal regions were studied, therefore, in a number of lizards, to see whether any clue could be obtained as to how the tympanic cavity and membrane of mammals might have evolved. This was done with the realization that only those features could be relied on which might reasonably be considered primitive and to have been present also in early mammal-like reptiles. In fact, the lizard ear, compared with that of other modern reptiles such as turtles and crocodiles, appears to retain in most respects a relatively simple organization. The Eustachian tube communicates directly with the pharynx as in the embryo (Fig. 2); the position of the tympanic membrane behind and below the quadrate and above the retro-articular process of the articulare is similar to its presumed site in pelycosaurs and other primitive synapsids; and Westoll (1943) and Parrington (1955) have shown that the cartilaginous processes of the extra-

stapes, which are so prominent in lizards, have their equivalents in all tetrapods, living and extinct.

It was found that in lizards, in front of the opening of each Eustachian tube and at a somewhat lower level, there is a lateral extension of the pharyngeal cavity which forms a deep groove on each side below the jaw (Fig. 3A). Embryologically this groove is formed as a recess of the hyoid pouch, antero-ventral to the precursor of the tympanic cavity, so that eventually it separates the skeletal elements, with their musculature, of the mandibular and hyoid arches. At its posterior end each groove fades out below the Eustachian tube, from which it is separated by the retro-articular process of the articulare and the attached adductor mandibulae internus muscle (Fig. 2). The lining epithelium of the groove consists of ciliated columnar



Fig. 2. *Acanthodactylus* post-natal. Transverse section showing middle ear structures in a lacertid lizard. The adductor mandibulae internus is ventral to the Eustachian tube and dorsal to the submandibular sulcus. The chorda tympani crosses the roof of the tympanic cavity.

and goblet cells and is arranged in folds similar to that of the floor of the pharynx and oesophagus adjoining it. Deep to the epithelium are many capillaries derived mainly from the tracheo-laryngeal branches of the pulmonary arteries, which also supply the pharyngeal floor. It is proposed to call this lateral extension of the pharynx the *sulcus pharyngis submandibularis*, or submandibular (pharyngeal) sulcus.

The appearance and relations of the submandibular sulcus are so like those of the developing tubotympanic recess of mammals (Fig. 3B), that there would seem good reason to suppose that, if such a structure were present in synapsids, it might well have played a part in the genesis of the mammalian middle ear cavity. The function of the submandibular sulcus in lizards is almost certainly to facilitate distension of the pharynx, particularly in the pulsatile movements which occur with pharyngeal

respiration. Drummond (1946) produced anatomical and experimental evidence that the pharynx and oesophagus, apart from assisting in ventilating the lungs, have themselves an active respiratory role in lizards, up to 10 % of the resting CO_2 being eliminated across their walls. It is clear from Fig. 3A that, when the hyobranchial apparatus is raised by the geniohyoid and mylohyoid muscles, the walls of the submandibular sulcus will be opposed, but descent of the floor of the pharynx,

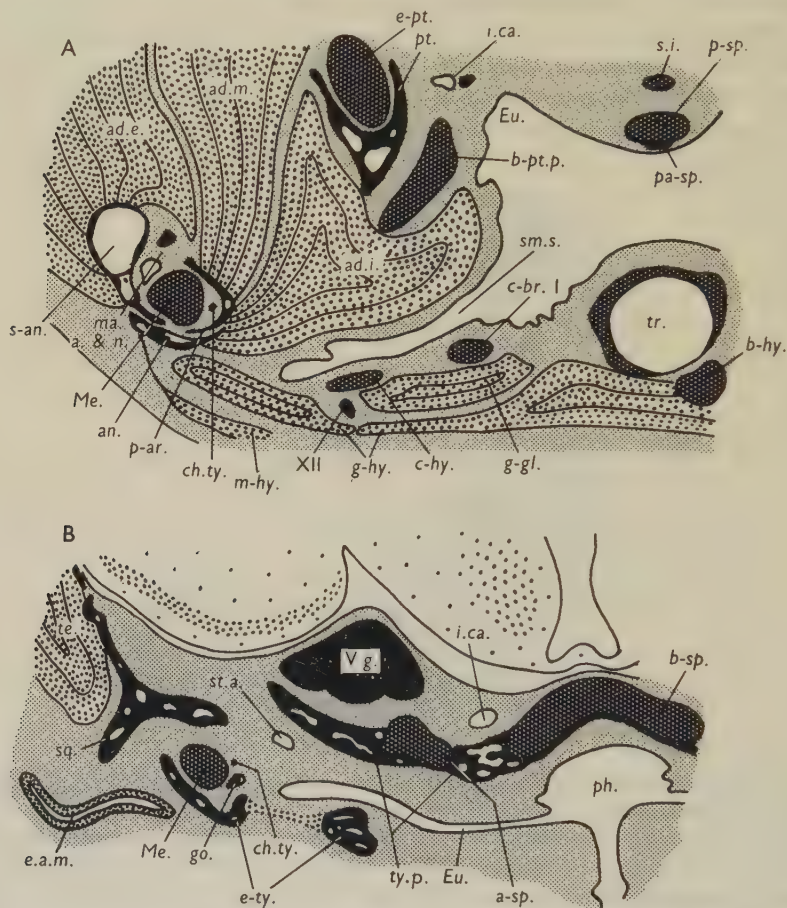


Fig. 3. (A) *Psammodromus* post-natal. Transverse section showing the relation of the submandibular sulcus to lower jaw structures in a lacertid lizard. (B) *Potamogale* embryo 16 mm. head length. Comparable transverse section through the Eustachian tube in an Insectivore. The ectochondral ossification below the basisphenoid and alisphenoid (tympanic process) is probably the equivalent of the reptilian parasphenoid.

necessarily accompanied by some unfolding or stretching of the subgular skin, will result in each sulcus opening out, thereby increasing both the volume of the cavity of the pharynx and the area of pharyngeal epithelium exposed to the air contained in it. This accessory respiratory mechanism may well have been utilized by early synapsids. Parrington (1934) suggested that the possession by some therapsids of a distinct thorax may have indicated that they had developed diaphragmatic

breathing. This would have enabled them to dispense with the less efficient methods required by their predecessors, and the submandibular sulcus could then be converted to other uses.

Some positive evidence that early mammal-like reptiles possessed a submandibular sulcus is provided by Sushkin's observation (1927) that in primitive (non-sphenacodontid) pelycosaurs and in captorhinomorphs there was a smooth, broad impression on the ventro-medial face of the back of the mandible. Sushkin believed that this impression received a diverticulum of the tympanic cavity, but it seems much more likely that it was caused by the submandibular sulcus, which could have made contact with any part of the back of the mandible not covered by muscle. It is significant that Sushkin also regarded the impression as an initial stage of the notch caused by the reflected lamina of the angulare which characterized all therapsids and advanced (sphenacodontid) pelycosaurs. A well-developed notch could hardly have failed to accommodate the submandibular sulcus, so long as the pterygoideus musculature (adductor mandibulae medius and internus) did not extend down on to the reflected lamina. Apart from its probable relation to the outer wall of the submandibular sulcus, which has not previously been realized, the reason for the existence of a reflected lamina has been disputed. Romer & Price (1940) supposed that its function was in fact to increase the insertion of the pterygoid muscles; Parrington (1955), on the other hand, associated it, in gorgonopsids at least, mainly with the masseter. If Romer & Price are correct in their interpretation, the submandibular sulcus could not have entered the notch of the angulare directly from the medial side, although it might perhaps have grown into it from behind.

Probably the reflected lamina of the angulare was first developed as a broad muscular process for the insertion of the masseter on its lateral surface, and incidentally supported the submandibular sulcus on the inner aspect of the notch. At some point, however, before the emergence of mammals, the masseteric attachment must have migrated forwards on to the dentary, leaving the angulare free. The reflected lamina then underwent reduction: in advanced cynodonts it formed a narrow bar somewhat medial to the body of the dentary. A similar reduction occurring in the actual ancestors of mammals would have permitted this vestigial muscular process to become a rudimentary ectotympanic. At the same time, the submandibular sulcus, now regressive as an organ of respiration, would have been available and suitable by virtue of its relation to the reflected lamina for conversion to the service of hearing. First, however, the outer wall of the sulcus must make contact with the surface. This would not have been difficult even if the old eardrum had disappeared as Watson believed, needing only that the mylohyoid and geniohyoid muscles should migrate forwards along the mandible (Fig. 2A). If, on the other hand, the reptilian drum was never entirely lost, contact could have been achieved by a downgrowth of its cuticular layer towards the submandibular sulcus in the depths of an external auditory meatus.

Could such an event have occurred during the history of cynodonts? An external auditory meatus is generally believed to have been present in the advanced cynodont *Gomphognathus*, in a deep groove at the back of the jugal process of the squamosal, and may have occupied a similar position, as Parrington (1955) has shown, in earlier therapsids. The groove in the squamosal may have partly lodged some type of

cartilaginous pinna, and its base marks the postero-superior limit of what would have been the attachment of the tympanic membrane—in *Gomphognathus*, immediately below the squamoso-quadrato articulation. A membrane stretching from here downwards and ventrally to the notch of the angulare, as reconstructed by Watson (1951, 1953), would have been fantastically large and unwieldy. It could not have contributed significantly to the transmission of sound by air-conduction via the articulare, whilst that bone still formed an integral part of the lower jaw; nor via the extra stapedial process, since the antero-inferior extension would have been cut off from the rest of the drum by the retro-articular process, and also by the depressor mandibulae which, according to Parrington (1955), was still present and inserted into the retro-articular process in *Gomphognathus*. More probably, the tympanic membrane in all cynodonts was a relatively small structure still occupying the primitive position opposite the outer end of the stapes, below and behind the quadrato, and above the retro-articular process of the articulare.

If these assumptions are correct, the mammalian type of tympanic membrane must have originated very late in therapsid history, little in advance of the establishment of a sound-conducting ossicular chain. Direct evidence of the process, therefore, is unlikely to be forthcoming until more is known of the anatomy of the ictidosauroids—the therapsid group transitional to mammals. In the present state of knowledge it is impossible to say more than that the mammalian drum, supported by a tympanic annulus, *could* have been formed as a result of the submandibular sulcus approaching either the skin surface or more probably the cuticular layer of the original tympanic membrane, and to indicate further changes in bony and soft tissues which would have led therefrom to the formation of the middle ear cavity and Eustachian tube of mammals.

Quite early in synapsid evolution the retro-articular process of the articulare tended to become deflected downwards, in connexion, as Parrington (1955) has argued, with the function of the depressor mandibulae. This, together with shortening of the postdentary portion of the mandible would have brought the angulare closer to the external auditory meatus, if present. Growth of the latter in a downwards and forwards direction, lateral to the retro-articular process, could have caused an extension of the cuticular layer to be applied to the outer wall of the submandibular sulcus, the two epithelia being supported circumferentially by the angulare and its reflected lamina. In this way, part of the drum would be brought below the level of the lower jaw (Fig. 4A). If by this time the depressor mandibulae had regressed or lost its original insertion, the old supramandibular and the new submandibular parts of the drum would be continuous below the retro-angular process. The extension might then have served to augment the conduction of sound to the extrastapedial process.

Externally, the enlarged drum would be formed by a single epithelial sheet, derived from the cuticular layer of the external auditory meatus; but its inner layer would be compound, being formed by the walls of the original tympanic cavity and the submandibular sulcus meeting on either side of the retro-articular process. After the regression of the depressor mandibulae these two diverticula could become continuous at their openings into the pharynx, below and behind the retro-articular process. They may have remained partly distinct so long as the articulare and

quadrate formed the functional jaw-joint. The original tympanic cavity could then continue to make hearing possible by enveloping the stapes and extrastapes, while the primary auditory purpose of the submandibular sulcus, which would have suffered from the disadvantage of moving with the lower jaw so long as the angulare was part of it, would be to contribute the mucosal layer to the new portion of the

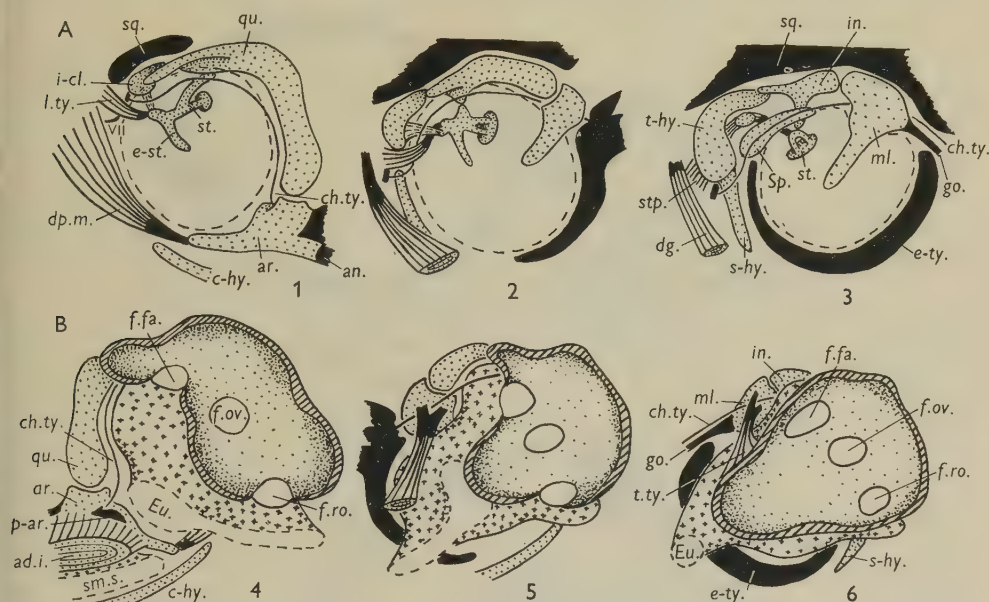


Fig. 4. Semi-schematic diagrams indicating how the mammalian middle ear could have evolved from a reptilian precursor. Membrane bones are shown black and chondral structures shaded with a dotted stipple. (A) Right ear, lateral view. The position of the tympanic membrane is marked by a broken line. (1) Lacertilian condition. The tympanic membrane is supported above and in front by the quadrate, below by the retro-articular process, and behind by the depressor mandibulae. (2) Hypothetical intermediate stage, with the extra-stapes retained, the articular freed from the depressor mandibulae, and the tympanic membrane extending to the reflected lamina of the angulare. (3) Mammalian condition. Shrapnell's membrane (not shown) occupies the area above the chorda tympani, whose course relative to the tympanic membrane proper is unchanged. (B) Right ear, medial view, showing the cut outer wall only of the auditory capsule, and omitting the tegmen tympani and entotympanic. The mouths of the Eustachian tube and submandibular sulcus are marked by broken lines, and the walls of the tympanic cavity and submandibular sulcus are stippled with crosses. (4) Lacertilian condition. The Eustachian tube opens above and the submandibular sulcus below the retro-articular process and the adductor mandibulae internus. (5) Hypothetical intermediate stage, in which the submandibular sulcus has made contact with the tympanic membrane and has become confluent with the original tympanic cavity. (6) Mammalian condition. The narrowed Eustachian tube opens in the site of the former submandibular sulcus. The tensor tympani is shown with a double tendon embracing the chorda tympani, as in bats.

drum. Eventually, the two would form a common middle ear cavity, in which the articular and quadrate become enclosed.

The partial closing off of this cavity from the pharynx was probably linked with the development of the cochlea. In the evolutionary line leading to mammals there must have been an improvement not only in sound conduction but also in the

perceptive apparatus of hearing, whereby the original papilla basilaris of primitive reptiles became greatly elongated to form an organ of Corti. To accommodate the latter, the pro-otic grew out antero-ventrally, and in so doing would occupy the space taken up by the wide mouth of the original Eustachian tube. In this way the tympanic cavity would acquire a new medial wall in the previously deficient lower

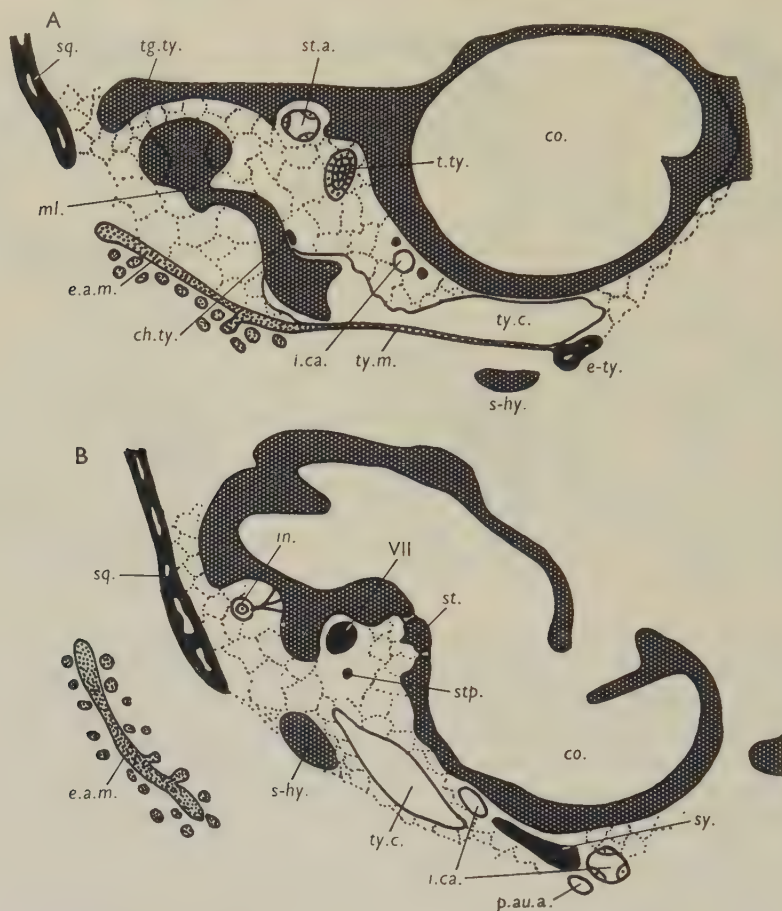


Fig. 5. *Elephantulus* embryo, 27.5 mm. Transverse sections showing middle ear structures in a primitive eutherian mammal. (A) Level of the malleo-incudal joint. The tympanic membrane is unattached along its latero-superior margin. The tympanic cavity has not yet invaded the attic region, which is filled by mesenchyme. (B) Level of the footplate of the stapes, behind the tympanic membrane, passing through the posterior tip of the tympanic diverticulum. The short process of the incus is seen in the fossa incudis. The internal carotid artery and the accompanying cranial sympathetic trunk are entering the middle ear.

part which communicated directly with the pharynx (cf. Figs. 2 and 5A). The original Eustachian tube would become obliterated, and the pharyngeal opening of the middle ear cavity be limited to the narrowed mouth of the submandibular sulcus, which would then represent the definitive Eustachian orifice of the nasopharynx (Fig. 4B).

The mammalian middle ear, therefore, compared with its reptilian precursor, has an enlarged lateral wall due to the extension of the eardrum, and an enlarged medial wall due to the growth of the cochlea. It acquires a new roof from the tegmen tympani growing out from the periotic, and a new floor from the entotympanic. The membranous lining has, according to the foregoing argument, a twofold origin. The submandibular component, derived from the submandibular sulcus, forms the Eustachian tube and the part of the middle ear between the promontory of the cochlea and the segment of the drum below and in front of the handle of the malleus. The supramandibular component, derived from the original tympanic cavity, forms the rest of the middle ear chamber; it no longer connects directly with the pharynx, owing to the growth of the cochlea closing its medial wall, but communicates with the submandibular component below the handle of the malleus. Structures such as the secondary tympanic membrane and the carotico-stapedial arterial trunk, which were dorsal to the old Eustachian tube, find themselves in the roof of the tympanic cavity well behind the pharynx (Fig. 5B).

The following further modifications must occur to bring the middle ear to the full mammalian state. The retro-articular process, already in the position of a manubrium mallei, will take on its shape and function. The adductor mandibulae internus, now a tensor tympani and no longer a masticatory muscle, will occupy the roof of the new Eustachian tube, since that structure is morphologically a submandibular derivative. The angularis will be freed from the lower jaw and become applied to the squamosal. Further growth of its reflected lamina will cause it to give attachment, not only to the new extension of the drum, but also to the back of its original postero-superior part, which was originally supported by the belly of the depressor mandibulae. The transformation to an ectotympanic is then complete. Of the other postdentary membrane bones, the surangularis is probably lost or reduced to the occasional ossiculum accessorium mallei, and the pre-articularis converted to the anterior process of the malleus (see Discussion).

According to the present theory, the primitive eardrum of reptiles is represented in mammals not by Shrapnell's membrane but by the morphologically supra-mandibular portion of the pars tensa—that is, the segment behind and above the manubrium mallei. Shrapnell's membrane is regarded as a new formation in mammals—an upward extension of the drum proper which, with the squamosal, forms the lateral boundary of the attic region or epitympanic recess, accommodating the bodies of the malleus and incus. Behind the epitympanic recess is the fossa incudis, enclosing the short process of the incus: the latter is probably derived from the posterior flange of the quadrate, which originally supported the tympanic membrane from above (Figs. 4A, 5B, 6). The epitympanic recess is partially separated from the rest of the middle ear chamber by the anterior and posterior malleolar folds. These form inward projections of the mucosal layer of the drum, and may be relics of the process by which the articulare and quadrate became incorporated into the tympanic cavity. The posterior malleolar fold corresponds in position to the outer part of the roof and anterior wall of the reptilian middle ear, formed by the quadrate. On this interpretation, the chorda tympani has not changed its course relative to the tympanic membrane during the evolution of mammals. Its relation to the posterior malleolar fold between the back of the pars tensa and

Shrapnell's membrane is the precise mammalian equivalent of its situation in lizards, below and behind the quadrate in the roof and anterior wall of the middle ear (Fig. 2).

The chorda tympani in reptiles retaining a middle ear runs dorsal to the cartilaginous extrastapes. In mammals, where an efficient sound-conducting apparatus is provided by the malleus and incus, a direct connexion between the stapes and the eardrum is no longer desirable: the extrastapedial process accordingly becomes vestigial, or perhaps disappears. It is necessary in this respect to consider the status of the cartilages of Paauw and Spence which, although commonly regarded as extrastapedial remnants, have given rise to some controversy. These small elements chondrify much later than other middle ear structures, and do not appear to occur

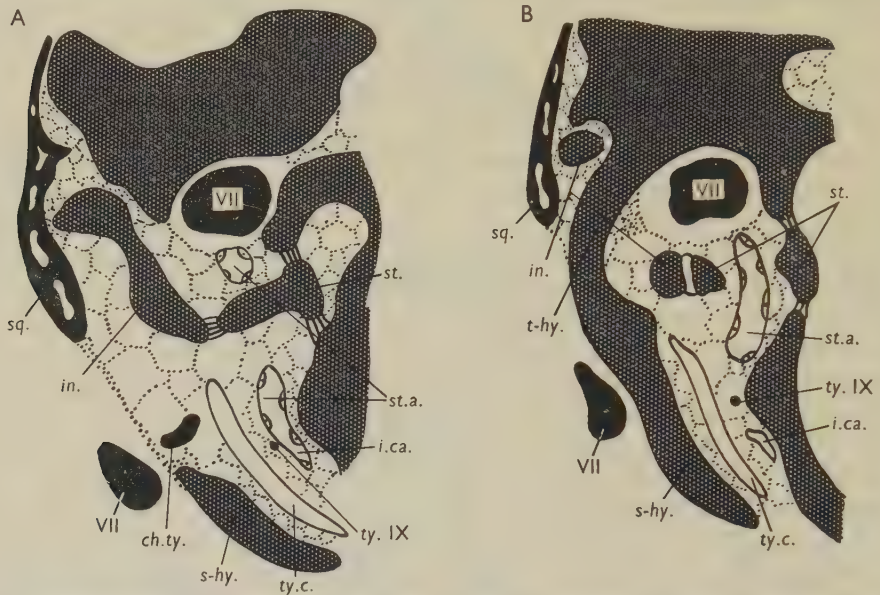


Fig. 6. *Potamogale* embryo, 16 mm. head length. Transverse sections illustrating the formation of the fossa incudis between the squamosal laterally and the tympanohyal medially. In (A) the chorda tympani is hooking round the stylohyal, and in (B), which is more caudal, the stapedial artery is piercing the stapes.

in every mammalian group. Paauw's cartilage, situated in the tendon of the stapedius muscle, has not been found in Insectivora. Spence's cartilage is laid down in mesenchyme adjacent to, and partly enclosing, the chorda tympani as it follows the posterior malleolar fold (Fig. 7A). McClain (1939) did not report it as such in *Didelphys*, but described a cartilage lateral to the malleus which he homologized, tentatively and most improbably, with the membranous surangulare. His account and figures suggest that this structure may have been the cartilage of Spence.

Olson (1944), following van der Klaauw (1923), regarded Spence's cartilage as homologous with the processus internus or quadrate process of the stapes. Westoll (1944) rejected this view, on the reasonable grounds that the point of contact between the stapes and quadrate is likely to have remained constant, so that the processus internus must form the head of the stapes in mammals. He considered

Spence's cartilage to be equivalent to the 'distal (tympanic) portion' of the extrastapes—that is, the processus inferior, and Paauw's cartilage to the 'proximal', horizontal or hyostapedial portion. Findlay (1943) had previously identified the latter with Spence's cartilage, and the processus inferior with the manubrium mallei. Findlay's view, as Westoll pointed out, is at variance with the strong embryological and palaeontological evidence for regarding the manubrium mallei as a derivative

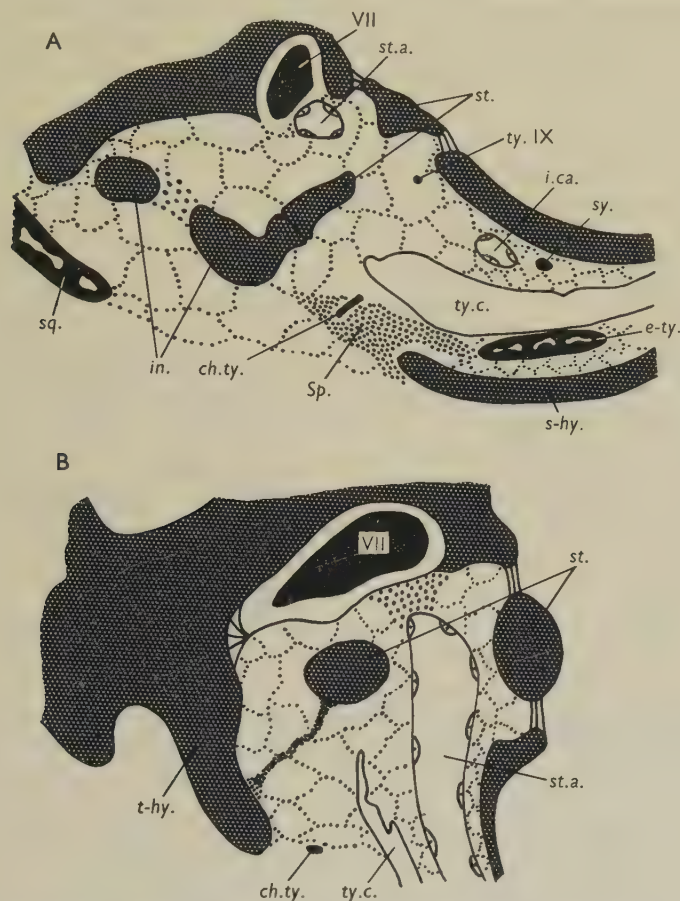


Fig. 7. (A) *Elephantulus* embryo, 27.5 mm. Transverse section showing the posterior end of Spence's cartilage at a blastemata stage, forming around the chorda tympani. Spence's cartilage was chondrified in a 47 mm. embryo of *Elephantulus*. (B) Neonatal mouse. Transverse section showing the occurrence of a ligamentous connexion between the stapes and the tympanohyal.

of Meckel's cartilage and equivalent to the retro-articular process of the articulare. On the other hand, Westoll's interpretation, although fitting the general position of the cartilages, has the disadvantage of requiring the extrastapedial process to have been fragmented into two pieces while failing to explain the associations of the fragments with the stapedius muscle and the chorda tympani.

In those reptiles which have lost the tympanic membrane, the stapes becomes firmly fixed to the quadrate and the extrastapes disappears. If, then, the cartilages

of Paauw and Spence are indeed extrastapedial vestiges, their persistence in mammals is evidence against the tympanic membrane having been lost and reformed in ancestral synapsids. It is by no means certain, however, that these elements are atavistic structures. Since the Eustachian cartilage seems to have been developed by mammals with no known reptilian precursor, it is possible that the cartilages of Paauw and Spence should also be regarded as mammalian specializations, and their late appearance in ontogeny would support this view. The significance of each may be functional rather than morphological: Paauw's cartilage acting as a sesamoid, and Spence's cartilage supporting the upper part of the pars tensa of the tympanic membrane.

If, on the other hand, the cartilages of Paauw and Spence do represent parts of the reptilian extrastapedial apparatus, they must be considered in terms of the comparative embryology of the hyoid arch skeleton. In early embryonic stages

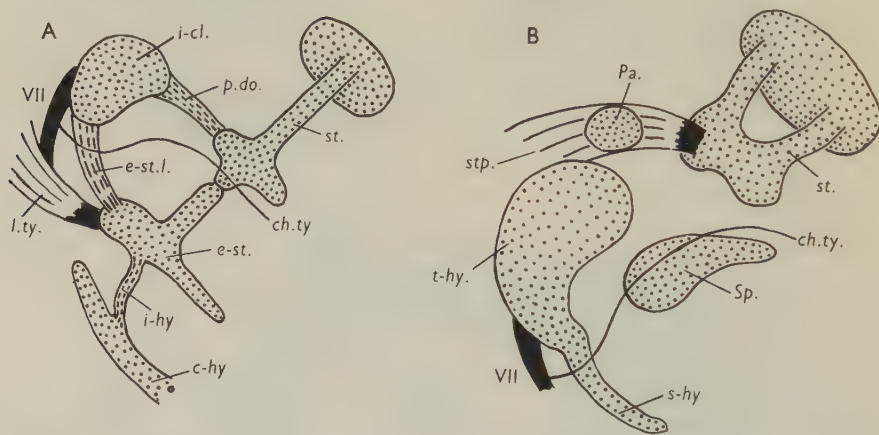


Fig. 8. Diagrams illustrating dorsal hyoid derivatives related to the right ear in a lizard (A) and a mammal (B). Whether the cartilages of Paauw and Spence can be equated to structures shown in (A) is uncertain.

of *Lacerta* a loop from the dorsal (epihyal) end of the hyoid blastema is raised up from the horizontal or hyostapedial portion and gains attachment to the crista parotica of the auditory capsule. The apex of this loop enlarges to form the intercalary cartilage between the quadrate and the squamosal; the outer or 'laterohyal' limb of the loop becomes the extrastapedial ligament joining the processus inferior or tympanic process of the extrastapes to the intercalary, and the inner limb persists for a while as the processus dorsalis or suprastapedial process and then regresses (Fig. 8A). These epihyal derivatives become separated from the ceratohyal as a result of degeneration of the intervening interhyal element. *Sphenodon* differs from the lacertid condition in that the interhyal and both limbs of the hyoid loop chondrify (see Versluys, 1904; Wyeth, 1924). The chorda tympani in lizards, in spite of Brock's statement and figure to the contrary (1932), hooks round both the processus dorsalis and the extrastapedial ligament. In mammals the hyoid cartilage makes contact with the crista parotica through the tympanohyal (Fig. 8B).

Goodrich (1916, 1930), following Versluys, homologized the mammalian tympanohyal with the reptilian intercalary. His figures indicate the processus dorsalis, hyostapes and interhyal as forming a connexion between the intercalary and the ceratohyal corresponding to the mammalian stylohyal. If this were so, there might be a case for interpreting Spence's cartilage, on account of its close relation to the chorda tympani, as a laterohyal remnant, the homologue of the extrastapedial ligament. Goodrich, however, appears to have been unduly influenced by the failure of the laterohyal to chondrify in *Lacerta*. In synapsids little is known of the fate of the dorsal end of the hyoid arch, but there is no reason why it should have conformed precisely to the commonest lacertilian pattern. According to Parrington (1955), the processus dorsalis of synapsids was probably still attached to the stapes, and may have helped in its support. As, in the ancestors of mammals, the stapes decreased in size, the processus dorsalis would no longer have been required as a supporting structure, and may consequently have undergone reduction. On the other hand, the lateral limb of the original hyoid loop may have become chondrified, forming a stylohyal for the attachment of tongue and throat muscles. On this view, part at least of the mammalian stylohyal or styloid process corresponds to the extrastapedial ligament (and interhyal) of typical lizards. The tympanohyal is the equivalent of the intercalary: in forming the medial wall of the fossa incudis (Fig. 6) it occupies a position comparable to that of the intercalary in lizards medial to the posterior flange of the quadrate (Fig. 4A). The blastema of Spence's cartilage appears to develop in the interval between the tympanohyal and the stapes, and may therefore possibly represent a processus dorsalis. It is interesting that in a newborn mouse, where no cartilage of Spence is formed, the dorsal tip of the tympanohyal, which in mice is characteristically recurved forwards, was found to be connected to the stapes by a distinct ligamentous strand running medial to the chorda tympani (Fig. 7B). This strand is not in the position of a hyostapes, but could be interpreted as a vestigial processus dorsalis.

The only link between Paauw's cartilage of mammals and the reptilian extrastapes is provided by the stapedius muscle, in whose tendon the cartilage forms. There is some reason for equating the stapedius with the laxator tympani muscle of geckonid and agamid lizards, in spite of a changed relation to the facial nerve (Goodrich, 1930). The laxator tympani is inserted into the back of the tympanic process of the extrastapes. If Paauw's cartilage is indeed a vestigial structure, it would seem reasonable, following van der Klaauw (1923) and Olson (1944), to regard it as the homologue of the extrastapedial process as a whole, forming with the tendon of insertion of stapedius a reduced version of the original cartilaginous connexion between the stapes and the tympanic membrane (Fig. 8A).

DISCUSSION

It is concluded that the tubotympanic recess of mammals is formed in the position of a submandibular diverticulum of the pharynx present as a respiratory device in modern reptiles and probably, in view of Sushkin's observations, also possessed by ancestral synapsids. The pars tensa of the mammalian tympanic membrane is an enlarged version of the reptilian drum, which has acquired a submandibular extension and become embraced by the angulare. The pars flaccida or Shrapnell's membrane

is a new upgrowth, above the original site of the upper end of the quadrate, towards the squamosal. It may get an attachment to the ectotympanic when that bone becomes completely annular. An attempt has been made to show how the reptilian type of middle ear could have been transformed without any 'saltations' into its mammalian successor during the stages of evolution following the release of the reflected lamina of the angulare and the retro-articular process of the articulare from muscle attachments.

The theory is claimed to be preferable in certain important respects to those which have been put forward previously. First of all, it fits the facts of morphology. The Eustachian tube bears the correct relation to the tensor tympani—ventral to the muscle in mammals. It accounts for the obliquity of the mammalian external auditory meatus and Eustachian tube, the former inclining downwards and forwards, and the latter upwards and backwards, towards the eardrum. At the same time, a reason is offered for the changed relation of the internal carotid and the fenestra rotunda to the tympanic cavity. The position of the chorda tympani between the pars tensa and Shrapnell's membrane is explained. The course of this nerve is not absolutely constant throughout vertebrate history. In lizards it may pierce or pass above the adductor mandibulae internus, and Gray (1953) has emphasized its variable relation in mammals to the tendon of the tensor tympani. In geckonid and pygopodid lizards there is no processus dorsalis, and the chorda tympani runs directly forward in front of the middle ear. McClain (1939) has described how in early embryos of *Didelphys* the nerve is pulled through the blastema of the stylohyal from lateral to medial. Nevertheless, other things being equal, it is an advantage not to have to postulate a shift across the tympanic membrane during the evolution of mammal-like reptiles.

Secondly, some of the obscurities in the palaeontological story are clarified. The dilemma due to the later cynodonts possessing an angulare with, apparently, ectotympanic characters, when a tympanic membrane attached to it could have had little or no auditory function, is resolved without introducing the extra hypothesis of a 'resonator' derived from the middle ear. There is no need to devise a complicated extrastapedial process which would reach to the notch of the angulare in early forms, nor to suppose that late forms redeveloped a lost tympanic membrane. If the ancestors of mammals did at one stage lose their capacity to hear by air conduction, yet possessed a submandibular sulcus, it would have been possible for them to reform a tympanic cavity entirely from it. If, as appears more likely, however, the original tympanic cavity was retained, and the mammalian middle ear chamber had a twofold origin in the manner suggested, the presence of a submandibular sulcus makes it a little easier to understand how the angulare, articulare and quadrate came to free themselves from the jaws and be taken up into the middle ear.

Thirdly, the theory is in keeping with what can be inferred from embryology; if it is true, there is in the developmental history of the middle ear in mammals a fair degree of recapitulation. The relation between the tubotympanic recess and the ectotympanic in the embryo insectivore *Potamogale* (Fig. 3B) is the same as that which it has been supposed the submandibular sulcus bore to the angulare in cynodonts. This does not necessarily mean that cynodonts were ancestral to mammals, but simply that in the angulare, as in other features, they exhibit an

intermediate condition which may also have occurred in actual mammalian ancestors. The subsequent steps which have been postulated in the theory are reflected in the ontogeny of the mammalian drum, as it appears in Insectivora such as *Elephantulus*. The external auditory meatus grows down from above the manubrium mallei to meet the tubotympanic recess above the lower arm of the ectotympanic (Fig. 5A): this corresponds to the suggested migration of the meatus in the final stages of therapsid history to meet the submandibular sulcus above the reflected lamina of the angulare. The developing tympanic membrane is at first unattached at its upper margin; the external auditory meatus meets the squamosal, and the tubotympanic recess invades the attic region so late as to lend support to the view that Shrapnell's membrane was phylogenetically a late acquirement.

Assuming it to be true that the lacertilian pharynx and middle ear retains many ancestral reptilian features, and that mammals have evolved away from the primitive pattern in the manner described, it becomes possible to compare equivalent regions of the ear in lizards and primitive eutherian mammals such as the Insectivora. Towards the back of the ear, a transverse section through the fenestra ovalis in *Elephantulus* is retropharyngeal and passes through the point of entry of the internal carotid and the cranial sympathetic trunk into the auditory capsule (Fig. 5B). A comparable section in a lizard, passing through the Eustachian tube, illustrates how the cochlea has usurped the place of the latter in mammals (Fig. 2). Anteriorly, the mammalian Eustachian tube connecting with the pharynx at the level of the alisphenoid (Fig. 3B) has obvious affinities with the lacertilian submandibular sulcus at the comparable level of the basipterygoid process and the epipterygoid (Fig. 3A). Here, too, the mammalian goniale—that is, the membranous ossification forming the long anterior process of the malleus—can be seen to bear just the same relation as the reptilian pre-articulare to Meckel's cartilage and the chorda tympani. In spite of the doubts expressed by Olson (1944) and Westoll (1944), Gaupp's original claim (1911a) seems justified that these bones are homologous. It is true that in Insectivora the goniale may be partly fused with the ectotympanic: but the two elements are quite distinct for the greater part of their extent, and a much more intimate fusion occurs between the pre-articulare and angulare amongst lizards in the Geckonidae.

The significance of the cartilages of Paauw and Spence—whether they are to be regarded as extrastapedial and suprastapedial vestiges or as mammalian neomorphs developed in response to some functional need—depends in part on whether the tympanic membrane of mammals is considered to have evolved anew or from a reptilian precursor. It is less likely that any cartilaginous appendages of the reptilian stapes would have persisted if the eardrum had once been lost. This point may never be settled beyond question, but the balance of evidence is on the whole against the view that the tympanic membrane ever disappeared entirely in ancestral premammals, even though it may have been lacking in some Synapsida. Many of the best-known and best-preserved fossils are large forms, probably off the direct mammalian line. Moreover, we know from the anatomy of lizards that, in one reptilian group at least, it is possible even within the same family for the middle ear and drum to be lost in some genera and retained in others. The embryological appearances are certainly consistent with the tympanic cavity of mammals having

been developed entirely from the submandibular sulcus. On the other hand, the mammalian middle ear could, as has been shown, have arisen as a result of the original tympanic cavity becoming merged with the submandibular sulcus, without loss of the drum. The latter alternative avoids the need to invoke parallelism to account for a structure such as the mammalian stapedius, which resembles fairly closely its reptilian counterparts in crocodiles and lizards, and which would have been unlikely to survive total regression of the tympanic membrane.

The view expressed here, that the Eustachian tubes of mammals and reptiles are not strictly equivalent, has also formed part of an evolutionary theory of the middle ear developed by Tumarkin (1955, latest statement). No support, however, can be given to his basic tenet that absence of the middle ear is invariably a primitive character, and that this organ has been and is being evolved separately by all tetrapods which possess one. Tumarkin's conclusions have been severely criticized by Parrington (1949), and Vaughn (1955) has pointed out that the concept of ear reduction is unavoidable unless hypotheses are to be multiplied beyond all the evidence. Although it is often difficult to distinguish simplicity of structure which is truly primitive from that which is regressive, an almost indisputable example of the latter is provided by the ear of the little-known lizard *Aprasia*. This small, burrowing pygopodid, unlike other members of its family, lacks a tympanic membrane and Eustachian tube. Further, there is no stapes. In the place of the footplate of the stapes, the fenestra vestibuli supports a membrane which, since the fenestra rotunda is closed, probably functions as a secondary tympanic membrane during bone-conduction of sound via the quadrate. Absence of the stapes in an adult lizard can hardly be interpreted except in terms of degeneracy.

SUMMARY

1. Objections are raised to the theory that the middle ear cavity of mammals was evolved from a diverticulum of the original reptilian tympanic cavity and that Shrapnell's membrane is a relic of the reptilian eardrum.

2. A lateral diverticulum of the floor of the pharynx is described in lizards and named the submandibular sulcus. It is claimed that this diverticulum may have been present in Synapsida and have played a part in respiration.

3. The submandibular sulcus is shown to possess relations to lower jaw structures comparable to those of the tubotympanic recess in mammalian embryos.

4. It is suggested, therefore, that the middle ear cavity of mammals may have evolved either from the submandibular sulcus alone or, more probably, by incorporation of the original tympanic cavity into the submandibular sulcus.

5. Accepting the latter alternative, the pars tensa of the tympanic membrane of mammals is regarded as an extension of the original reptilian drum, and Shrapnell's membrane as a new formation consequent upon the retreat of the bodies of the malleus and incus into the middle ear.

6. The cartilages of Paauw and Spence are interpreted as being either mammalian neomorphs or, possibly, vestiges of respectively the extrastapedial and supra-stapedial process.

7. The tympanohyal and stylohyal of mammals are probably homologous with the intercalary, together with a laterohyal element of the dorsal end of the hyoid

arch skeleton forming the extrastapedial ligament in typical lizards, rather than with the processus dorsalis and hyostapedial portion of the extrastapes.

8. A condition of the auditory capsule is described in the lizard *Aprasia*, which appears to prove that absence of the middle ear, in this tetrapod at least, is a regressive rather than a primitive character.

I wish to thank Prof. J. D. Boyd for the loan of the *Elephantulus* embryos and for helpful criticism, and Mr F. R. Parrington for having discussed with me many of the points raised in this paper. The lizard material illustrated in the figures was collected in Algeria, with the aid of a grant awarded by the trustees of the Durham Fund, King's College, Cambridge.

REFERENCES

- BROCK, G. T. (1932). Some developmental stages in the skulls of the geckos, *Lygodactylus capensis* and *Pachydactylus maculosa*, and their bearing on certain important problems in lacertilian craniology. *S. Afr. J. med. Sci.* **29**, 508-532.
- DRUMMOND, F. H. (1946). Pharyngo-oesophageal respiration in the lizard, *Trachysaurus rugosus*. *Proc. zool. Soc. Lond.* **116**, 225-228.
- FINDLAY, G. H. (1943). The development of the auditory ossicles in the elephant shrew, the tenrec and the golden mole. *Proc. zool. Soc. Lond.* **114**, 91-99.
- GAUPP, E. (1911*a*). Beiträge zur Kenntnis des Unterkiefers der Wirbeltiere. I. Der Processus anterior (Folli) des Hammers der Säuger und das Goniale der Nichtsäuger. *Anat. Anz.* **39**, 97-135.
- GAUPP, E. (1911*b*). Beiträge zur Kenntnis des Unterkiefers der Wirbeltiere. II. Die Zusammensetzung des Unterkiefers der Quadrupeden. *Anat. Anz.* **39**, 433-473.
- GAUPP, E. (1913). Die Reichertsche Theorie. *Arch. Anat. Physiol., Lpz.*, Suppl. Bd. (1912), pp. 1-416.
- GOODRICH, E. S. (1916). The chorda tympani and middle ear in reptiles, birds and mammals. *Quart. J. micr. Sci.* **61**, 137-160.
- GOODRICH, E. S. (1930). *Studies on the Structure and Development of Vertebrates*. London: Macmillan.
- GRAY, O. (1953). The chorda tympani. *J. Laryng.* **67**, 128-138.
- GREGORY, W. K. (1951). *Evolution Emerging*. New York: Macmillan.
- KEIBEL, F. & MALL, F. P. (1912). *Manual of Human Embryology*, 2. Philadelphia and London: J. P. Lippincott.
- VAN DER KLAUW, C. J. (1923). Die Skelettstückchen in der Sehne des Musculus stapedius und nahe dem Ursprung der Chorda tympani. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **69**, 32-83.
- MCCLAINE, J. A. (1939). The development of the auditory ossicles of the opossum (*Didelphys virginiana*). *J. Morph.* **64**, 211-265.
- OLSON, E. C. (1944). Origin of mammals based upon cranial morphology of the therapsid suborders. *Spec. Pap. geol. Soc. Amer.* **55**, 1-136.
- PALMER, W. R. (1913). Note on the lower jaw and ear ossicles of a foetal *Perameles*. *Anat. Anz.* **43**, 510-515.
- PARRINGTON, F. R. (1934). On the cynodont genus *Galesaurus*, with a note on the functional significance of the changes in the evolution of the theriodont skull. *Ann. Mag. nat. Hist.* (10), **13**, 38-67.
- PARRINGTON, F. R. (1946). On the cranial anatomy of cynodonts. *Proc. zool. Soc. Lond.* **116**, 181-197.
- PARRINGTON, F. R. (1949). Remarks on a theory of the evolution of the tetrapod middle ear. *J. Laryng.* **63**, 580-595.
- PARRINGTON, F. R. (1955). On the cranial anatomy of some gorgonopsids and the synapsid middle ear. *Proc. zool. Soc. Lond.* **125**, 1-40.
- PARRINGTON, F. R. & WESTOLL, T. S. (1941). On the evolution of the mammalian palate. *Phil. Trans. B*, **230**, 305-355.

- ROMER, A. S. & PRICE, L. I. (1940). Review of the Pelycosauria. *Spec. Pap. geol. Soc. Amer.* **28**, 1-538.
- SUSHKIN, P. P. (1927). On the modifications of the mandibular and hyoid arches and their relations to the brain-case in the early Tetrapoda. *Paläont. Z.* **8**, 263-321.
- TUMARKIN, A. (1955). On the evolution of the auditory conducting apparatus: a new theory based on functional considerations. *Evolution*, **9**, 221-243.
- VAUGHN, P. P. (1955). The Permian reptile *Araucoscelis* restudied. *Bull. Mus. comp. Zool.* **113**, 305-467.
- VERSLUYS, J. (1904). Entwicklung der Columella auris bei den Lacertiliern. Ein Beitrag zur Kenntniss der schalleitenden Apparate und des Zungenbeinbogens bei den Sauropsiden. *Zool. Jb. 2. Anat. Ont.* **19**, 107-188.
- WATSON, D. M. S. (1951). *Palaeontology and Modern Biology*. New Haven: Yale University Press.
- WATSON, D. M. S. (1953). The evolution of the mammalian ear. *Evolution*, **7**, 159-177.
- WESTOLL, T. S. (1943). The hyomandibular of *Eusthenopteron* and the tetrapod middle ear. *Proc. Roy. Soc. B*, **131**, 393-414.
- WESTOLL, T. S. (1944). New light on the mammalian ear ossicles. *Nature, Lond.*, **154**, 770-771.
- WESTOLL, T. S. (1945). The mammalian middle ear. *Nature, Lond.*, **155**, 114-115.
- WYETH, F. J. (1924). The development of the auditory apparatus in *Sphenodon punctatus*; with an account of the visceral pouches, aortic arches and other accessory structures. *Phil. Trans. B*, **212**, 259-368.

EXPLANATION OF FIGURES

Probable homologies

Reptilian	Mammalian
adductor mandibulae externus	temporalis and masseter
adductor mandibulae medius	pterygoid muscles
adductor mandibulae internus	tensor tympani and tensor palati
depressor mandibulae	digastricus posterior and stylo-hyoid
laxator tympani	stapedius
angulare (reflected lamina)	ectotympanic
pre-articulare	goniale
articulare	malleus
retro-articular process	manubrium mallei
quadrate	incus
intercalary	tympanohyal
extrastapedial ligament	stylohyal (part)
pterygoid	pterygoid
epipterygoid	alisphenoid
parasphenoid	tympanic process of basisphenoid

List of abbreviations

<i>a-sp.</i>	alisphenoid	<i>e-ty.</i>	ectotympanic
<i>ad.e.</i>	adductor mandibulae externus	<i>Eu.</i>	Eustachian tube
<i>ad.i.</i>	adductor mandibulae internus	<i>f.fa.</i>	foramen faciale
<i>ad.m.</i>	adductor mandibulae medius	<i>f.ov.</i>	fenestra ovalis
<i>an.</i>	angulare	<i>f.ro.</i>	fenestra rotunda
<i>ar.</i>	articulare	<i>g-gl.</i>	genioglossus
<i>b-hy.</i>	basihyobranchial	<i>g-hy.</i>	geniohyoid
<i>b-pt.p.</i>	basipterygoid process	<i>go.</i>	goniale
<i>b-sp.</i>	basisphenoid	<i>i.ca.</i>	internal carotid artery
<i>c-br. I</i>	ceratobranchial I	<i>i-cl.</i>	intercalary
<i>c-br. II</i>	ceratobranchial II	<i>i-hy.</i>	interhyal
<i>c-hy.</i>	ceratohyal	<i>in.</i>	incus
<i>ch.ty.</i>	chorda tympani	<i>l.h.v.</i>	lateral head vein
<i>co.</i>	cochlea	<i>l.ty.</i>	laxator tympani
<i>cr.p.</i>	crista parotica	<i>la.</i>	larynx
<i>dg.</i>	digastricus posterior	<i>m.ml.</i>	manubrium mallei
<i>dp.m.</i>	depressor mandibulae	<i>m-hy.</i>	mylohyoid
<i>e.a.m.</i>	external auditory meatus	<i>ma. a. & n.</i>	mandibular artery and nerve
<i>e-pt.</i>	epipterygoid	<i>Mc.</i>	Meckel's cartilage
<i>e-st.</i>	extrastapes	<i>ml.</i>	malleus
<i>e-st.l.</i>	extrastapedial ligament	<i>or.</i>	orbit

List of abbreviations

<i>ot.</i>	otic ganglion	<i>st.</i>	stapes
<i>p.au.a.</i>	posterior auricular artery	<i>st.a.</i>	stapedial artery
<i>p.do.</i>	processus dorsalis	<i>stp.</i>	stapedius
<i>p-ar.</i>	pre-articulare	<i>sy.</i>	cranial sympathetic trunk
<i>p-sp.</i>	presphenoid	<i>t.ty.</i>	tensor tympani
<i>Pa.</i>	Paauw's cartilage	<i>t-hy.</i>	tympanohyal
<i>pa-sp.</i>	parasphenoid	<i>t-ty.r.</i>	tubotympanic recess
<i>ph.</i>	pharynx	<i>te.</i>	temporalis
<i>pi.</i>	pinna	<i>tg.ty.</i>	tegmen tympani
<i>pt.</i>	pterygoid	<i>tr.</i>	trachea
<i>qu.</i>	quadrate	<i>ty.c.</i>	tympanic cavity
<i>r-ar.p.</i>	retro-articular process	<i>ty.m.</i>	tympanic membrane
<i>Re.</i>	Reichert's cartilage	<i>ty.p.</i>	tympanic process of basisphenoid
<i>s.i.</i>	subiculum infundibuli	<i>ty. IX</i>	tympanic branch of glosso- pharyngeal nerve
<i>s-an.</i>	surangulare	<i>Vg.</i>	trigeminal ganglion
<i>s-hy.</i>	stylohyal	<i>VII</i>	facial nerve
<i>sm.s.</i>	submandibular sulcus	<i>XII</i>	hypoglossal nerve
<i>Sp.</i>	Spence's cartilage		
<i>sq.</i>	squamosal		

THE DEVELOPMENT OF THE CIRCULATION IN THE SPLEEN OF THE FOETAL RABBIT

By O. J. LEWIS

Department of Anatomy, St Thomas's Hospital Medical School, London, S.E. 1

The nature of the splenic circulation has long been a matter of controversy. Weidenreich (1901), Robinson (1926), and MacNeal, Otani & Patterson (1927) believed the circulation to be of the so-called 'open type', with capillaries opening into the pulp, their endothelial walls becoming continuous with the reticulum. Mollier (1911), Thoma (1924), Knisely (1936*a, b*), and Peck & Hoerr (1951*a, b*) favoured the concept of a 'closed type' of circulation with the blood confined to endothelial-lined channels.

Mollier (1911) claimed that the sinuses were modified pulp spaces, varying in their degree of elaboration in different species, but always having incomplete walls with stomata. Robinson (1930) believed that, in the cat spleen, the sinuses commenced in the pulp, their walls being continuous with the reticulum. Mall (1903) believed in an 'open' circulation, but considered that there were channels of more direct and rapid flow in the pulp. Contraction of the capsule and trabeculae emptied the contents of most of the pulp spaces into the sinusoids, and confined blood flow to these channels. Mackenzie, Whipple & Wintersteiner (1941), using the transillumination method, reached similar conclusions.

Snook (1944, 1950) showed that spleens of different species varied in the degree to which sinuses were elaborated, and so were of 'sinusal' or 'non-sinusal' type. Significantly, he showed that the mouse and cat, which constituted the major part of Knisely's material, had spleens of 'non-sinusal' type.

Little work has been done on the development of the splenic circulation. Sabin (1912), Barta (1926) and Thiel & Downey (1921) considered that initially there was a closed capillary network of endothelial-lined vessels. From this network, according to Sabin, there developed tufts, or 'spherules', of wide-bored capillaries, which became the splenic pulp and sinusoids. Barta maintained that the closed capillaries developed ampullary dilatations which became increasingly permeable and poured blood into the surrounding pulp. Thiel & Downey (1921), and Ono (1930), described the sinuses as developing as slits in the mesenchyme of the splenic anlage.

MATERIALS AND METHODS

The earliest stages in splenic development were studied in Bouin-fixed rabbit embryos, serially sectioned at 10μ , and stained with haematoxylin and eosin. At later stages the spleens were removed and fixed, sectioned and stained as before. Embryos of 11–28 days' gestation were used.

In addition, the spleens of injected embryos of 21–27 days were studied, after clearing in methyl benzoate. At first indian ink, injected via the umbilical artery, was used, but only one injected spleen (22 days) was obtained from thirteen fully

injected embryos (Sabin found a similar difficulty). Later, a colloidal dye, Monastral Fast Blue BNVS Paste (supplied by I.C.I. Ltd.) was used, and about one-third of fully injected embryos were found to have injected spleens. Ten injected spleens were obtained by this method, as follows: 21 days, 1 spleen; 22 days, 1 spleen; 23 days, 1 spleen; 25 days, 1 spleen; 26 days, 5 spleens; 27 days, 1 spleen.

OBSERVATIONS

The splenic anlage was seen to arise as Holyoke (1936) observed, from mesoderm, largely from the multi-layered primitive mesothelium of the mesentery. Until 20 days it consists of dense, closely packed mesenchymal cells, and no capillaries can be seen.

The 21-day stage

At this stage the spleen is looser in texture, the cells becoming separated by communicating intercellular spaces, the walls of which are formed by the mesenchymal cells of the anlage (Pl. 2, figs. 8, 9). These spaces form a plexus of varicose, anastomosing channels within the spleen, as can be seen from injected specimens at this stage (Pl. 1, figs. 1, 2; Text-fig. 3). This plexus contains no blood cells at this stage. At a number of points, in series down the hilum of the organ, a branch of the splenic artery and a tributary of the splenic vein, join the plexus (Pl. 1, fig. 2; Text-fig. 3). At these points the endothelium of the arterial or venous vessels becomes continuous with the mesenchymal cells of the anlage which bound the tissue spaces (Pl. 2, fig. 9). In places, more discrete channels can be seen developing from those parts of the plexus which are immediately continuous with entering arterial branches—they are narrower, pursue a more definite course through the plexus, and are rather better filled with injection medium (Pl. 1, fig. 1; Text-fig. 3). Histological sections show that they are endothelial-walled capillaries developing from the plexus by differentiation of the mesenchymal walls into endothelium, the process proceeding some distance into the interior of the organ, from the point of entry of an artery at the hilum (Pl. 2, fig. 8).

The 23-day stage

The anlage has loosened further, and endothelial-walled capillaries of rather larger diameter can be found in it more frequently. The slit-like spaces between the mesenchymal cells have enlarged. Both the arterial capillaries and the slit-like spaces contain non-nucleated red cells, but prior to this stage no blood cells are observed in the splenic anlage.

Injection at this stage shows that differentiation of arterial capillaries from the primitive plexus has proceeded further to form a quite definite and elaborate system of endothelial-walled branches which are continuous with the remainder of the plexus, which consists of the enlarging slits in the mesenchyme. These slits are the primitive pulp spaces (Pl. 1, fig. 3) which drain into the veins leaving the hilum, the endothelium of the veins being continuous with the mesenchymal walls of the primitive pulp spaces.

The 25-day stage.

Arterial capillaries are more numerous, well defined, and of wider calibre, and their endothelial walls become continuous with the mesenchymal walls of the pulp spaces. Non-nucleated red cells pour from them into the pulp spaces which now contain more red cells (Pl. 2, fig. 10), and lymphoid sheaths are forming from the reticulum about the arterial vessels. More definite venous channels are commencing to develop from the venous end of the plexus of pulp spaces, by differentiation of the mesenchymal walls of the pulp spaces into endothelium.

Injection at this stage shows a more elaborate arborization of arterial capillaries, continuous with the wider-bored, dye-filled plexus of primitive pulp spaces. Veins and venous sinusoids are just commencing to differentiate from the plexus (Pl. 1, figs. 4, 5), the process proceeding into the interior from the hilum; thus, more discrete and larger channels of venous drainage are formed from the venous end of the plexus.

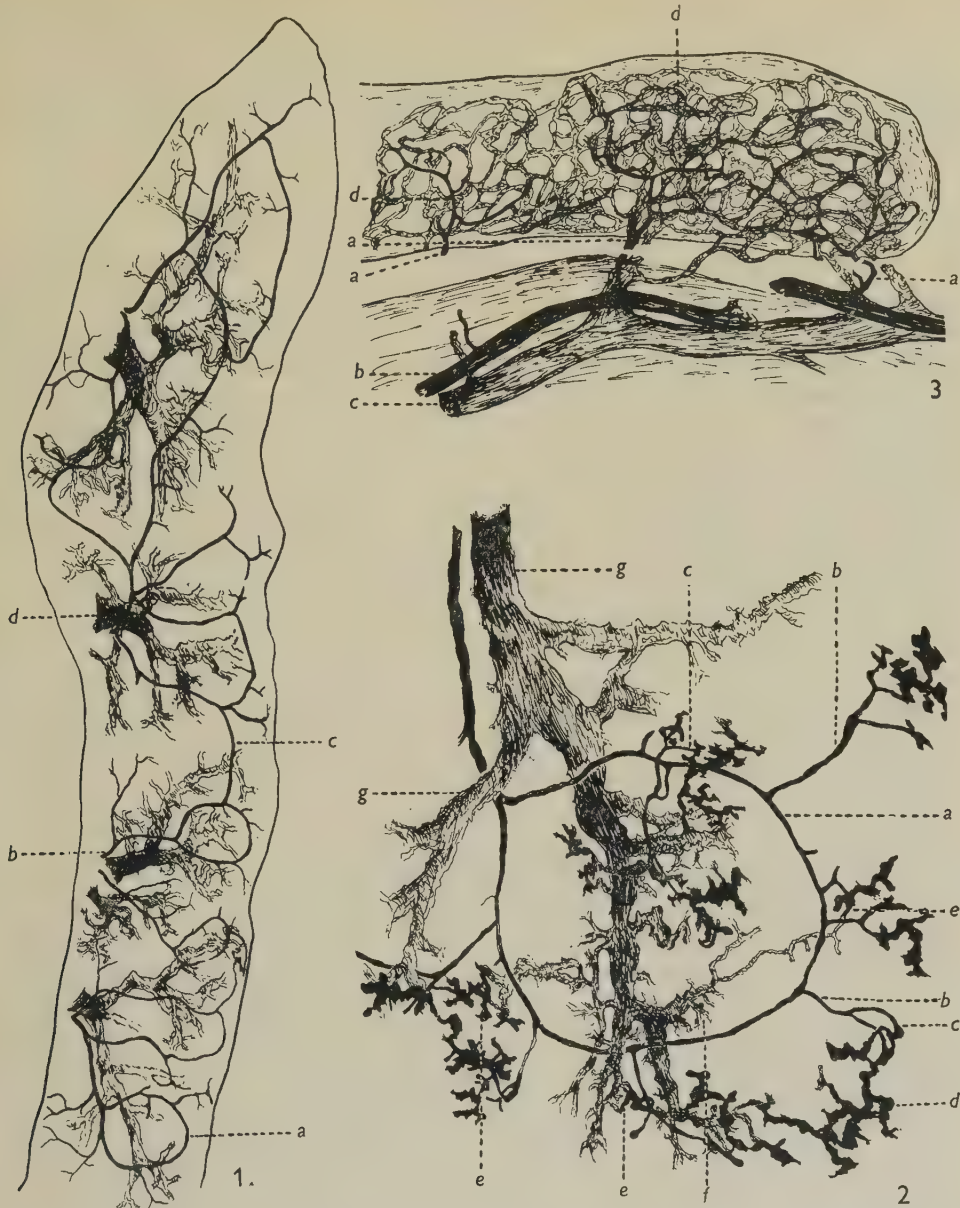
The 27-day stage

The arterial branches are more completely developed, terminating by funnel-shaped openings in the pulp, where the endothelium becomes continuous with the reticulum. Lymphoid sheaths are well marked (Pl. 2, figs. 11, 12).

Veins, both collecting veins and sinusoids, are developing further from the pulp spaces, their differentiation proceeding into the organ from the hilum. They drain non-nucleated red corpuscles (and nucleated red corpuscles, for haemopoiesis is established in the reticulum by this stage) from the pulp spaces (Pl. 2, figs. 13, 14).

From now until full term there is increasing elaboration of arteries from one end and veins from the other end of the original mesenchymal-lined plexus of channels. Pulp spaces, enlargements of the slits of the original plexus, continue to connect arteries and veins. There is also increasing haemopoiesis.

In a spleen of 27 days (Pl. 1, fig. 6; Text-fig. 1) the arteries are seen now to form a definite pattern consisting of a series of loops arising from the serially entering branches of the splenic artery. In some places the entering artery divides into branches which reunite to form a loop, in others the vessel joins with a neighbour to form an arcade, and occasionally the loop is almost, but not quite, complete, the branches communicating by a short channel in the pulp spaces. (The varying pattern is shown in Text-fig. 1.) From these loops arise branches, the endothelial walls of which finally become continuous with the mesenchymal walls of the pulp spaces. The pulp spaces are irregular, varicose and heavily injected with dye, and become continuous with more definitely outlined channels, the sinusoids, whose walls, however, also show extravasations of dye. The sinusoids are continuous with even more definitely walled collecting veins (Text-fig. 2; Pl. 1, fig. 7). It can also be seen (Pl. 1, fig. 6; Text-fig. 1) that the spleen can be subdivided into a series of vascular units, though the division is not absolute, for anastomoses between units do occur. This 27-day spleen was only partially injected, presumably due to contraction of the splenic artery and the developing muscle of capsule and trabeculae,



Text-fig. 1. Camera-lucida drawing of injected 27-day rabbit embryo spleen showing arterial loops and arcades. *a*, arterial loop; *b*, arterial branch entering at hilus; *c*, anastomotic branch between two arterial loops; *d*, vein leaving hilus. The smaller arterial branches, pulp spaces, and smaller venous tributaries are omitted for clearness.

Text-fig. 2. Enlarged camera-lucida drawing of arterial loop (*a*) in Text-fig. 1. *a*, arterial loop; *b*, branches of loop; *c*, arterial branches opening into pulp spaces; *d*, pulp spaces; *e*, pulp spaces opening into sinusoids; *f*, sinusoids; *g*, collecting vein.

Text-fig. 3. Camera-lucida drawing of injected 21-day rabbit embryo spleen showing the primordial plexus. Serially-arranged arterial branches (*a*), accompanied by venous branches, are entering the hilus; *b*, splenic artery; *c*, splenic vein; *d*, arterial branches differentiating from the plexus.

acting to confine blood flow to the most direct pulp channels (Mall, 1903; Mackenzie *et al.* 1941).

More completely injected spleens of about the same stage show a more elaborate arborization of venous sinusoids and a greater filling of pulp spaces, the whole spleen being engorged with injection material.

DISCUSSION

The primitive circulation of the spleen consists of a plexus of channels or tissue spaces within the mesenchyme, communicating by arterial and venous branches with the splenic artery and vein. The walls of this plexus are formed by the mesenchymal cells, and at this stage it contains no blood cells—presumably only plasma circulates in it. Thus the circulation, even at this early stage, is 'open', plasma bathing the reticular cells directly. Further development consists of a centripetal elaboration of part of the plexus into arteries and part into veins, by differentiation of the mesenchymal cells into endothelium, the intervening portion remaining as pulp spaces. A similar view has been taken of the development of vessels in other parts of the embryo, for it has been maintained that endothelial cells develop from cells of the reticular tissue and that diffusion passages are present before an organized circulation, the endothelial cells developing as a lining for these spaces later (Altschul, 1954).

As the spleen grows in size arterial branches develop from the primary plexus, the process proceeding into the interior of the anlage from the point of entry of an artery at the hilum. Blood does not now have to percolate through the organ from the hilum, for it is distributed throughout the growing spleen by the arterial branches. That part of the plexus following these arterial capillaries increases the calibre of its component channels by further loosening of the reticulum, thus creating the primitive pulp spaces which are walled by reticular cells. Non-nucleated red blood corpuscles now appear in the arterial capillaries and the primitive pulp spaces.

By the 25-day stage that part of the plexus (*i.e.* primitive pulp spaces) draining into the veins at the hilum shows development into more definite venous channels. The process penetrates into the interior of the organ from the hilum, establishing a venous system draining the various parts of the enlarging spleen. The walls of the larger veins nearest the hilum are apparently complete and there is little extravasation of injection medium; the smaller branches or venous sinusoids form an elaborate arborization with apparently less complete walls. The sinuses are somewhat elaborated pulp spaces in which the bounding reticular cells are orientated in the line of flow of the blood (Mollier, 1911). The sinusoids in turn communicate with the varicose, irregular, pulp spaces into which the arteries drain. More direct channels exist through this system of pulp spaces and minimal injection, probably caused by contraction of the organ, tends to confine injection medium to these channels. The extent to which sinusoids develop from the pulp would appear to vary in different species (Snook, 1950).

As the arterial branches continue to elaborate they form a series of loops and arcades from which the terminal branches supplying the pulp are derived. Sometimes the loops are incomplete, being then completed by pulp spaces. This appears

to be a mechanism in the foetus, assuring that all the branches opening into the developing pulp will carry blood at about the same pressure, since the pressure around the circuit will be uniform. If, on the other hand, branches arose serially off a stem, some parts of the pulp would receive blood at high pressure, and become engorged, at the expense of neighbouring regions supplied with blood at lower pressure.

The ampulla of Thoma (1924), and his *Zwischenstück* (intermediate part), the successive parts of the closed circulation he postulated in passing from artery to vein, are the more direct and enlarged pulp spaces.

The initial capillary network described by Sabin (1912) was the primitive plexus of vascular spaces in the mesenchyme. The so-called 'spherules of enlarged capillaries' (Sabin's precursor of the pulp) developing on the capillary network, were the widening primitive pulp spaces at the ends of the arteries, containing much injection medium. Thus, the earliest circulation of the spleen was originally considered to be in closed capillary loops and the proponents of an open circulation then postulated its opening up in various ways. The present study shows that the flow from the earliest stages is directly through the reticulum, though definite channels of flow do develop within it; these are the arteries, arterial capillaries, veins and venous sinusoids. Pulp spaces are interposed between arterial capillaries and venous sinusoids even in its fully developed form.

The fundamental plan of the circulation would appear, both in the embryo and in its definitive form, to be as it is in lower vertebrates (e.g. fish; Yoffey, 1928)—blood percolating through the meshwork of the reticulum and bathing the surface of the lymphoid tissue as it does so.

SUMMARY

1. The development of the circulation in the spleens of rabbit embryos was studied by histological sections and by the injection of embryos with Monastral Fast Blue BNVS Paste at various stages; the spleens of these injected embryos were cleared and mounted whole.

2. From the earliest stages the circulation is 'open', arterial vessels, veins, venous sinusoids and pulp spaces developing from a plexus of tissue spaces in the mesenchymal anlage.

3. The arteries in the late embryo form loops and arcades within the organ, from which the branches supplying the pulp arise. This ensures distribution of blood at equal pressures to different parts of the developing pulp.

I wish to express my thanks to Prof. D. V. Davies for constant encouragement and advice; to Mr J. S. Fenton, Mr A. L. Wooding, and Mr A. E. Clark for the photographs; and to Imperial Chemical Industries Ltd. for supplying the Monastral Fast Blue BNVS Paste used in this study.

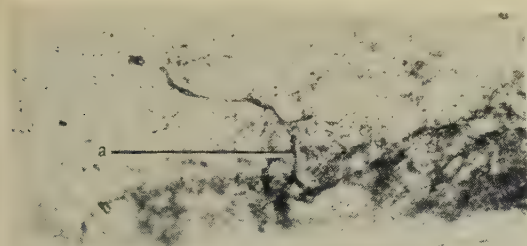
REFERENCES

- ALTSCHUL, R. (1954). *The Endothelium*, pp. 24–36. New York: The Macmillan Co.
- BARTA, E. (1926). Recherches sur le développement du système vasculaire de la rate et du foie. *C.R. Soc. Biol., Paris*, **94**, 1122–1124.
- HOLYOKE, E. A. (1936). The role of the primitive mesothelium in development of the mammalian spleen. *Anat. Rec.* **65**, 333–350.
- KNISELY, M. H. (1936*a*). Spleen studies. 1. Microscopic observation of the circulatory system of living unstimulated mammalian spleens. *Anat. Rec.* **65**, 23–50.
- KNISELY, M. H. (1936*b*). Spleen studies. 2. Microscopic observations of the circulatory system of living traumatized spleens, and of dying spleens. *Anat. Rec.* **65**, 131–147.
- MACKENZIE, D. W., JR., WHIPPLE, A. O. & WINTERSTEINER, M. P. (1941). Studies on the microscopic anatomy and physiology of living trans-illuminated mammalian spleens. *Amer. J. Anat.* **68**, 397–456.
- MACNEAL, W. J., OTANI, S. & PATTERSON, M. B. (1927). The finer vascular channels of the spleen. *Amer. J. Path.* **3**, 111–122.
- MALL, F. P. (1903). On the circulation through the pulp of the dog's spleen. *Amer. J. Anat.* **2**, 315–333.
- MOLLER, S. (1911). Über den Bau der Capillaren Milzvenen (Milzsinus). *Arch. mikr. Anat.* **76**, 608–657.
- ONO, K. (1930). Untersuchungen über die Entwicklung der menschlichen Milz. *Z. Zellforsch.* **10**, 573–603.
- PECK, H. M. & HOERR, N. L. (1951*a*). The intermediary circulation in the red pulp of the mouse spleen. *Anat. Rec.* **109**, 447–477.
- PECK, H. M. & HOERR, N. L. (1951*b*). The effect of environmental temperature changes on the circulation of the mouse spleen. *Anat. Rec.* **109**, 479–493.
- ROBINSON, W. L. (1926). The vascular mechanism of the spleen. *Amer. J. Path.* **2**, 341–356.
- ROBINSON, W. L. (1930). The venous drainage of the cat spleen. *Amer. J. Path.* **6**, 19–26.
- SABIN, F. R. (1912). The development of the spleen. In Keibel, F. & Mall, F. P., *Manual of Human Embryology*, Vol. 2. London: Lippincott.
- SNOOK, T. (1944). The guinea pig spleen. Studies on the structure and connections of the venous sinuses. *Anat. Rec.* **89**, 413–427.
- SNOOK, T. (1950). A comparative study of the vascular arrangements in the mammalian spleens. *Amer. J. Anat.* **87**, 37–77.
- TEHRER, J. & GRAHAME, T. (1930). The capsule and trabeculae of spleens of domestic mammals. *J. Anat., Lond.*, **65**, 473–481.
- THIEL, G. A. & DOWNEY, H. (1921). The development of the mammalian spleen with special reference to its haematopoietic activity. *Amer. J. Anat.* **28**, 279–339.
- THOMA, R. (1924). Der normale Blutstrom und die venöse Stauung in der Milz. *Virchows Arch.* **249**, 100–117.
- WEIDENREICH, F. (1901). Das Gefäßsystem der menschlichen Milz. *Arch. mikr. Anat.* **58**, 247–376.
- YOFFEY, J. M. (1928). A contribution to the study of the comparative histology and physiology of the spleen, with reference chiefly to its cellular constituents. *J. Anat., Lond.*, **63**, 314–344.

EXPLANATION OF PLATES

PLATE 1

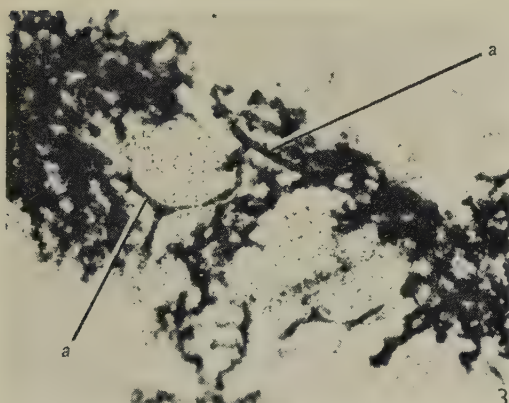
- Fig. 1. Spleen of 21-day rabbit embryo injected with Monastral Fast Blue. Part of vascular plexus is shown with an arterial branch (*a*) differentiating from it. $\times 140$.
- Fig. 2. Another region of the same spleen as fig. 1, showing arterial and venous branches (*b*) of splenic artery and vein (*c*) entering spleen to join up with plexus at (*e*). The plexus is rather poorly defined in the photograph owing to thickness of the specimen. $\times 140$.
- Fig. 3. Spleen of 23-day rabbit embryo injected with Monastral Fast Blue. Arterial branches (*a*) differentiated from the plexus are shown continuous with dye-filled primitive pulp spaces. $\times 100$.



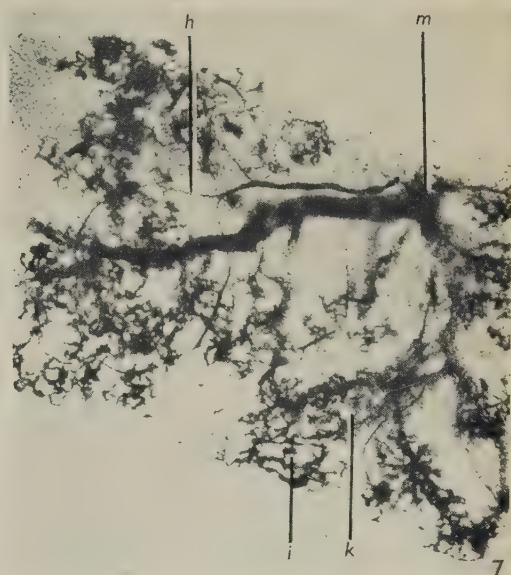
1



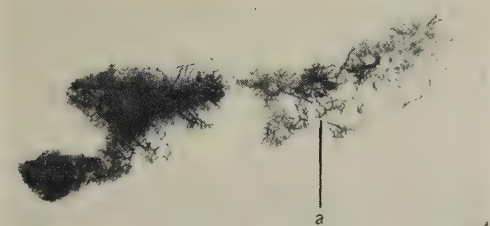
2



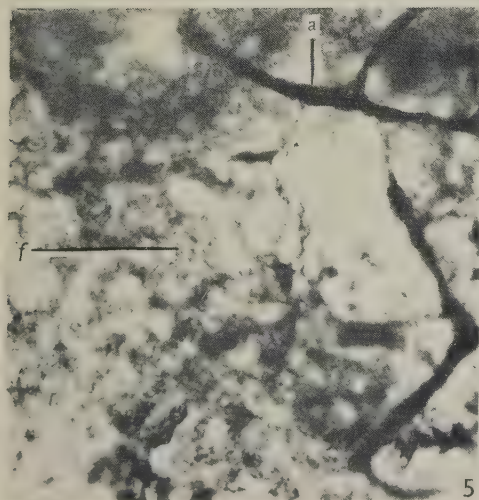
3



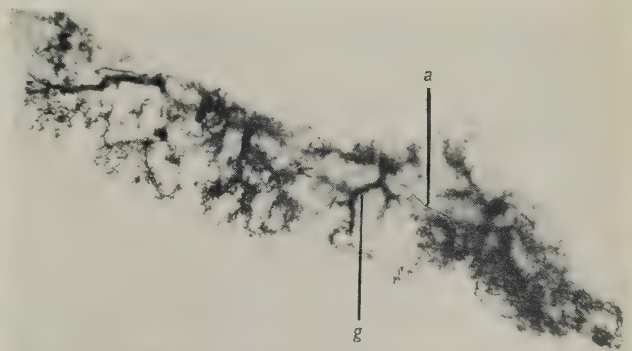
7



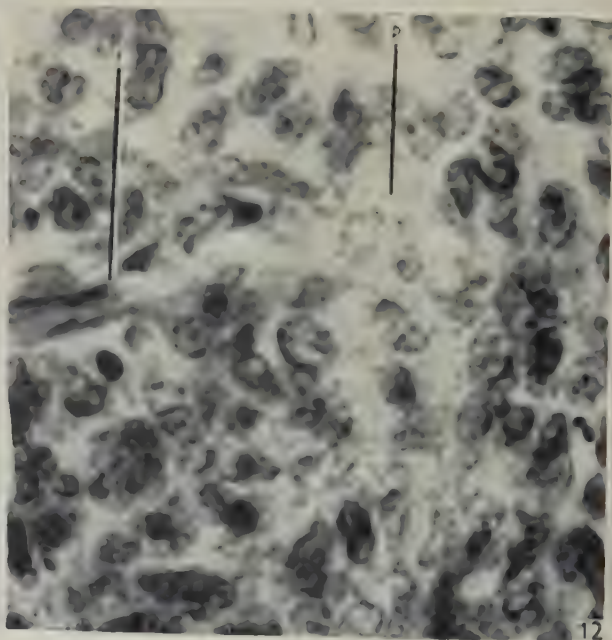
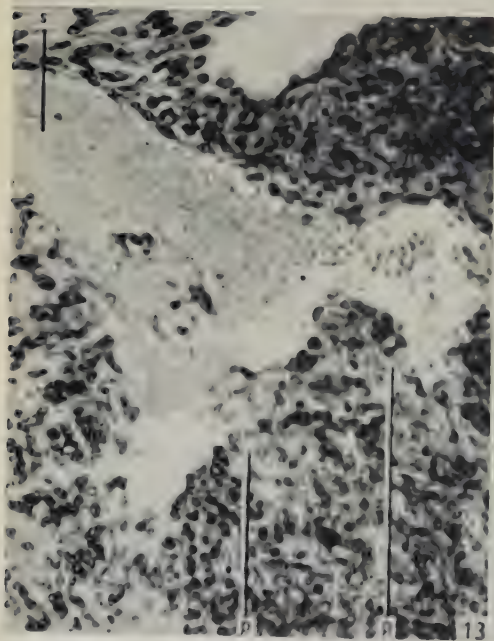
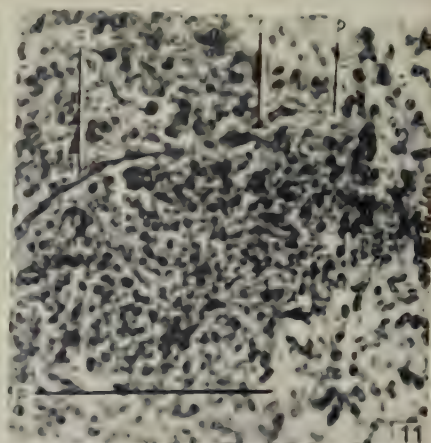
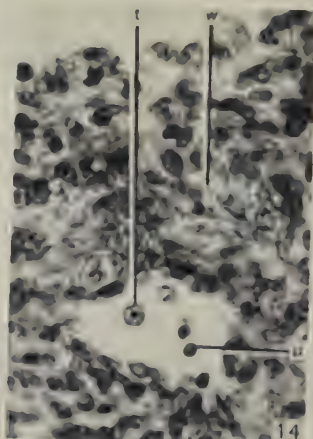
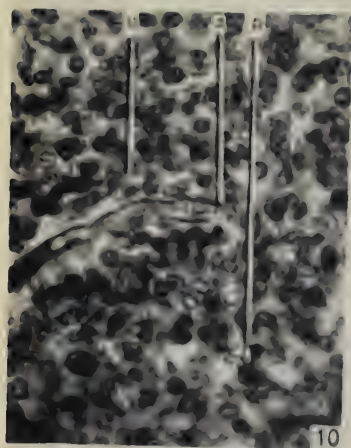
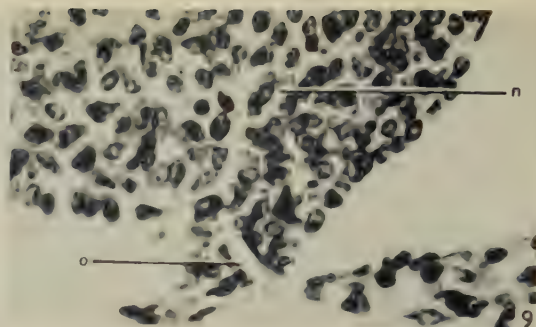
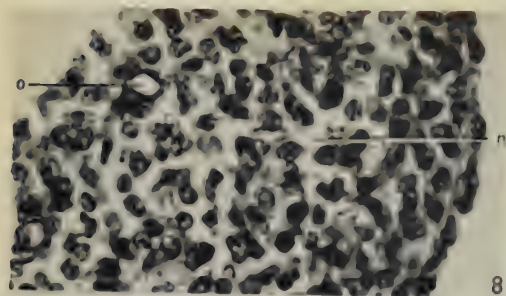
4



5



6



- Fig. 4. Spleen of 25-day rabbit embryo injected with Monastral Fast Blue. Arterial branches are shown in more elaborate arrangement (*a*). $\times 15$.
- Fig. 5. Same spleen as fig. 4. An arterial branch is shown (*a*) and a vein (*f*) is shown elaborating from the plexus and draining an arborization of sinusoids below, towards the hilus above. $\times 140$.
- Fig. 6. Spleen of 27-day rabbit embryo injected with Monastral Fast Blue. Arterial branches (*a*) are seen draining via pulp spaces and sinusoids into collecting veins (*g*). $\times 15$.
- Fig. 7. Same spleen as fig. 6. An arterial loop (*h*) is seen. The irregular pulp spaces (*i*) drain via sinusoids (*k*) into collecting veins (*m*) which leave the hilus. $\times 140$.

PLATE 2

- Fig. 8. Spleen of 21-day rabbit embryo, stained with haematoxylin and eosin. The dense mass of mesodermal cells presents connecting spaces (*n*). A very rare arterial capillary (*o*) can be found. $\times 460$.
- Fig. 9. Spleen of 21-day rabbit embryo, stained with haematoxylin and eosin. An arterial capillary (*o*) opens at the hilus into the meshwork of spaces (*n*) in the splenic anlage. $\times 460$.
- Fig. 10. Spleen of a 25-day rabbit embryo stained with haematoxylin and eosin. An arterial capillary containing adult-type red cells opens (*o*) into the reticulum. Adult type red cells are seen (*p*) in the primitive pulp spaces. A developing lymphoid sheath is seen (*q*). $\times 460$.
- Fig. 11. Spleen of 27-day rabbit embryo stained with haematoxylin and eosin. An arterial capillary (*o*) can be seen, invested with a well-developed lymphoid sheath, and opening (*r*) into the pulp, where red cells (*p*) are seen in the pulp spaces. A side branch of the arterial capillary can also be seen traversing the lymphoid sheath. $\times 300$.
- Fig. 12. An enlarged view of the opening of the arterial capillary seen in fig. 11. The endothelial cells are becoming continuous with the reticulum at (*r*) and red cells (*p*) are pouring into the pulp. $\times 1200$.
- Fig. 13. The same spleen as figs. 11 and 12. A vein (*s*) is seen at the hilus, draining red cells (*p*) directly from the pulp spaces of the reticulum. $\times 300$.
- Fig. 14. The same spleen as fig. 13. A collecting vein is seen in the interior of the spleen. It contains a basophil erythroblast (*t*) and a normoblast (*u*). Adult erythrocytes (*w*) can be seen in the pulp. $\times 460$.

ANATOMICAL FEATURES OF THE HUMAN RENAL GLOMERULAR EFFERENT VESSEL

BY J. P. SMITH

Department of Pathology, University of Manchester

There is in the literature a divergence of opinion on the existence of true muscular elements in the wall of the renal efferent glomerular vessel; one school believes that true muscle cells are present (Maximow & Bloom, 1942; Edwards, 1951), the other that they are absent (Volterra, 1926). A detailed review of the early literature was given by Bensley (1929).

The present paper shows that both views are, in fact, correct, the efferent vessel being either a muscular arteriole or an endothelial tube: anatomical variants of the efferent arteriole are also described giving histological confirmation of the injection studies of Boenig (1936) and Trueta, Barclay, Daniel, Franklin & Prichard (1947).

METHODS

Blocks of twenty-one adult, human, normal* kidneys taken at autopsy and fixed in Helly's fixative were used. A series of fifty consecutive 8μ sections from each block was mounted and stained by the periodic acid-Schiff technique. From these, drawings of portions of the arterial tree were constructed in which the efferent arterioles were identified and their distribution and anatomical structure noted.

The anatomical features seen in the glomerular efferent vessels in these twenty-one kidneys were searched for in serial ribbons of five sections each of kidneys from an unselected consecutive series of 678 autopsies (studied for another purpose, Smith, 1955); the series included therefore both normal and diseased kidneys. These sections were stained routinely by the P.A.S. technique; selected sections were stained by van Gieson's and Mallory's method.

OBSERVATIONS

The patterns of efferent vessels to be described have been found both in normal kidneys and in diseased kidneys; there appears no obvious relationship between any one particular type of efferent vessel and any one disease process.

Efferent vessels have been seen varying from well-formed arterioles with muscle cells in their walls (Pl. 1, fig. 1), which resemble in all respects those found in afferent arterioles, to little more than endothelial tubes some of which are large and solitary (Pl. 1, fig. 2), whilst others are numerous and small, resembling capillaries (Pl. 1, fig. 3). Well-formed arterioles usually proceed some distance from the glomerulus before breaking up into the intertubular capillaries, whereas vessels of endothelial-tube type usually break up more quickly.

* Normality means that the kidneys showed no macroscopic or microscopic evidence of nephrosclerosis. The hyaline arteriolar changes associated with age and hypertension were, of course, found in the afferent glomerular vessels.

Both muscular and endothelial types of efferent vessel may be found at all levels in the cortex but the muscular type predominates in the juxta-medullary glomeruli whose efferents form the chief blood supply of the medulla. Muscular efferents are more common than endothelial ones, but in some kidneys up to 40 % of efferents were of endothelial type.

In glomeruli with numerous capillary efferents, the efferent capillaries may arise directly from the glomerular capillaries (Pl. 1, fig. 3), but more usually the glomerular capillaries unite into a single endothelial vessel which then branches before penetrating Bowman's capsule (Pl. 1, fig. 4).

A small number of glomeruli are found in which two or more efferent vessels can be identified. Through the hilum of the glomerulus, shown in Pl. 1, fig. 5, passes a large efferent vessel (seen in consecutive sections to be of muscular type) with a leash of capillary efferents alongside it. A glomerulus having three main efferent vessels is shown in Pl. 2, figs. 6-8. In Pl. 2, fig. 6, the afferent arteriole is entering the glomerulus; in Pl. 2, fig. 7, one efferent vessel is emerging and in Pl. 2, fig. 8, two further efferent vessels are emerging from the hilum. Multiple efferent vessels are uncommon, not being found in more than 5 % of the glomeruli in any of my specimens; they occur at any level in the cortex.

Not all efferent vessels emerge from the hilum, though most do. Pl. 1, fig. 9, shows a glomerulus from which efferent vessels are emerging at diametrically opposing places. There is no evidence of any previous inflammatory disease in this glomerulus such as may have produced adhesions between the capillary tuft and the capsule.

COMMENT

It is unquestioned that there is muscle in the walls of afferent arterioles: sections stained by P.A.S., van Gieson's, and Mallory's methods show identical cytological structure of the walls of both afferent arterioles and some efferent vessels. It is therefore concluded that these efferent vessels contain muscular elements in their walls.

The type of efferent vessel bears no relationship to disease of either the glomerulus or the kidney. All types are equally common in both normal and hypertensive individuals but, from the study of the larger series of kidneys, I have the impression that multiple capillary efferents are more common in cases of diabetes mellitus. This impression may well be false as, in diabetic patients, pathological hyaline thickening of these small vessels makes them easier to see (Pl. 1, fig. 4).

The apparent absence of muscular elements from the wall of the endothelial-tube type of efferent vessel raises interesting problems about the control of filtration pressure in such glomeruli.

SUMMARY

1. The efferent vessel of the human renal glomerulus may be a muscular arteriole, a solitary wide endothelial tube or it may consist of numerous capillary vessels.
2. Occasionally more than one main efferent vessel emerges from a single glomerulus.
3. Rarely, efferent vessels emerge through Bowman's capsule at a point remote from the hilum of the glomerulus.

I wish to thank Prof. A. C. P. Campbell and Prof. G. A. G. Mitchell for reading and criticizing the manuscript.

REFERENCES

- BENSLEY, R. D. (1929). The efferent vessels of the renal glomeruli of mammals as a mechanism for the control of glomerular activity and pressure. *Amer. J. Anat.* **44**, 141-169.
- BOENIG, H. (1936). Beitrage zur Kenntnis der Vasa efferentia in der menschlichen Niere. *Z. mikr.-anat. Forsch.* **39**, 105-115.
- EDWARDS, J. G. (1951). The development of the efferent arteriole in the human metanephros. *Anat. Rec.* **109**, 495-501.
- MAXIMOW, A. A. & BLOOM, W. (1942). *A Textbook of Histology*. W. B. Saunders Co.
- SMITH, J. P. (1955). Hyaline arteriolosclerosis in the kidney. *J. Path. Bact.* **69**, 147-168.
- TRUETA, J., BARCLAY, A. E., DANIEL, P. M., FRANKLIN, K. J. & PRICHARD, M. M. L. (1947). *Studies of the Renal Circulation*, p. 71. Oxford.
- VOLTERRA, M. (1926). Quoted by Bensley.

EXPLANATION OF PLATES

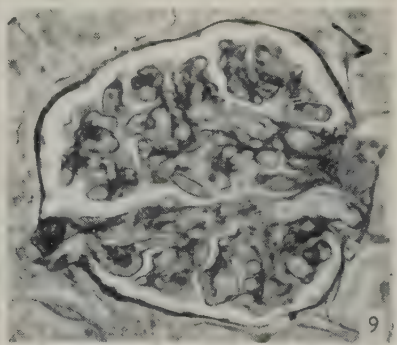
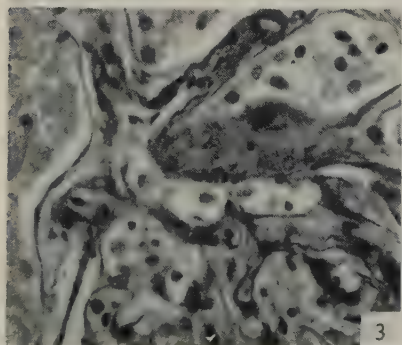
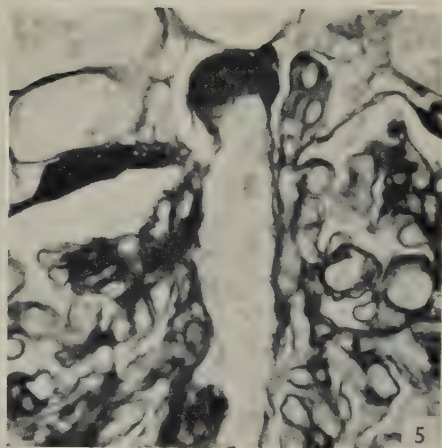
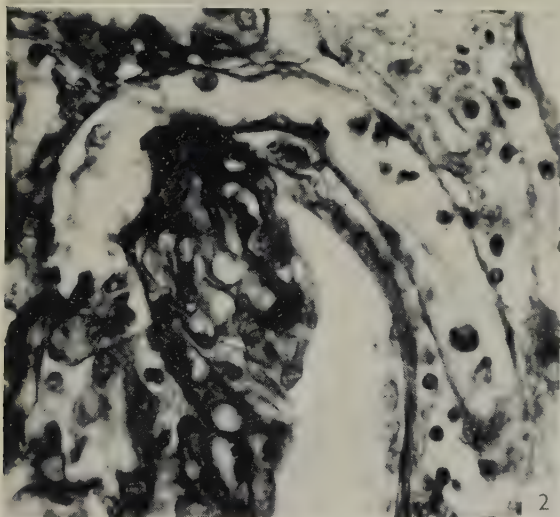
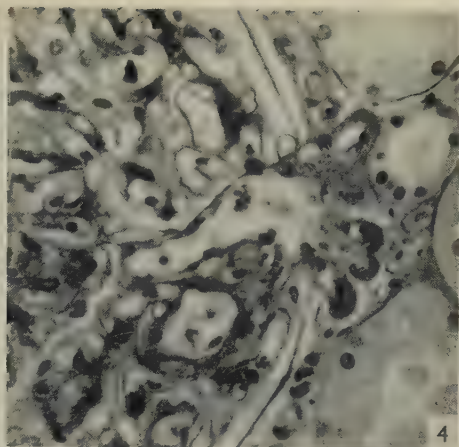
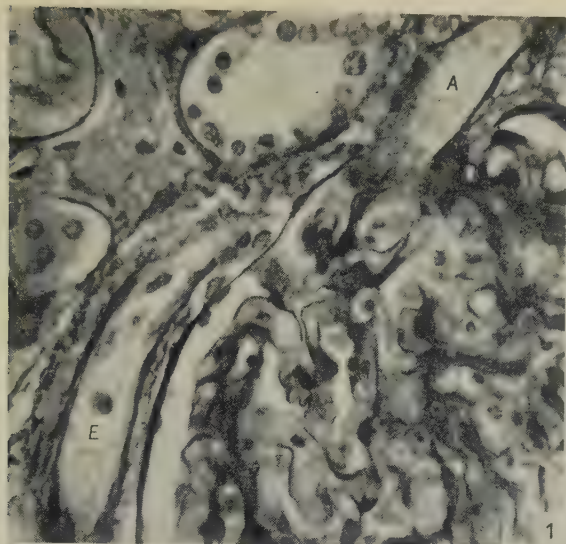
All the photomicrographs, with the exception of fig. 4, are of glomeruli found in normal kidneys from normotensive individuals. *A*=afferent arteriole; *E*=efferent arteriole.

PLATE 1

- Fig. 1. Renal glomerulus showing a muscular type of efferent arteriole to the left of the section and an afferent arteriole to the right. P.A.S. and haemalum, $\times 400$.
- Fig. 2. Renal glomerulus showing an endothelial-tube type of efferent vessel. This is the only efferent vessel emerging from this glomerulus. P.A.S. and haemalum, $\times 600$.
- Fig. 3. Glomerulus from the hilum of which numerous capillary efferent vessels are emerging. P.A.S. and haemalum, $\times 350$.
- Fig. 4. Glomerulus showing formation of a single efferent endothelial type of vessel which divides before it penetrates Bowman's capsule. Some of the efferent capillaries are hyalinized: this kidney was from a case of diabetes mellitus, but showed no evidence of Kimmelstiel-Wilson nephropathy. P.A.S. and haemalum, $\times 370$.
- Fig. 5. Glomerulus from the hilum of which emerge a large efferent vessel (seen in serial sections to be of muscular type) and a leash of efferent capillaries. P.A.S., $\times 470$.
- Fig. 9. A glomerulus showing one efferent vessel emerging at the hilum (to the right) and a further efferent vessel emerging at a point directly opposite. There is no evidence of disease in this glomerulus or in the rest of the kidney. P.A.S. and haemalum, $\times 220$.

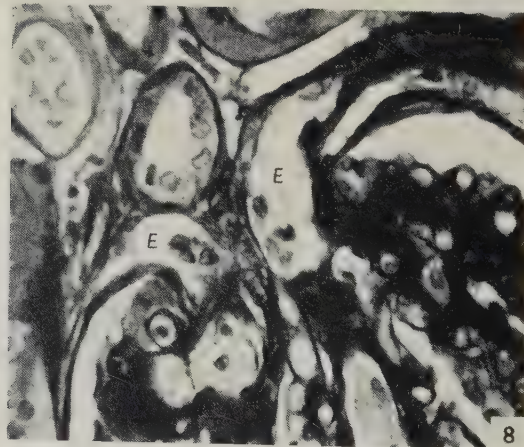
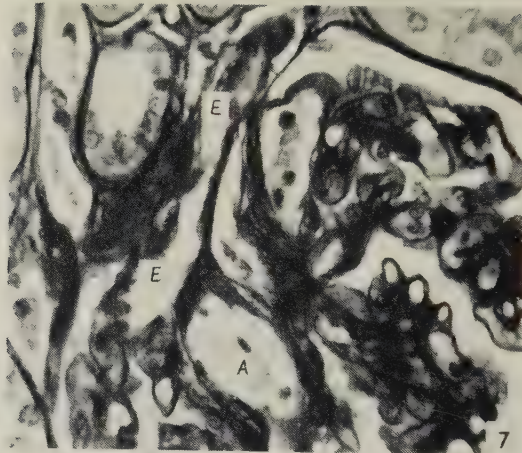
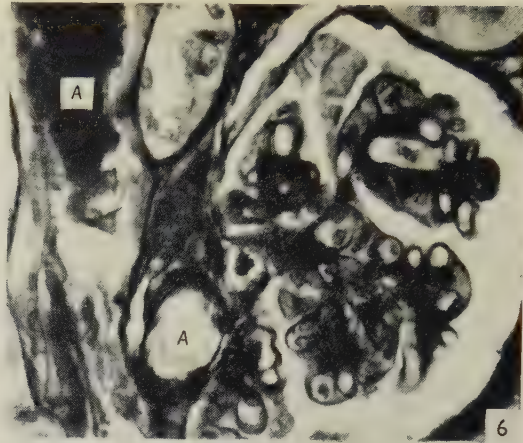
PLATE 2

- Fig. 6. Section 1 of a series of sections of a glomerulus. An afferent arteriole is entering the hilum. P.A.S. and haemalum, $\times 500$.
- Fig. 7. Section 5 of the same series of sections as in fig. 6. An efferent vessel is emerging at the hilum. P.A.S. and haemalum, $\times 500$.
- Fig. 8. Section 7 of the same series of sections as in figs. 6 and 7. Two further efferent vessels are emerging at the hilum. P.A.S. and haemalum, $\times 500$.



SMITH—FEATURES OF THE HUMAN RENAL GLOMERULAR EFFERENT VESSEL

(Facing p. 292)



SMITH—FEATURES OF THE HUMAN RENAL GLOMERULAR EFFERENT VESSEL

THE DISTRIBUTION OF HAEMOPOIETIC FOCI IN THE INFANTILE HUMAN LIVER

BY JOHN L. EMERY

Department of Pathology, Children's Hospital, Sheffield

During a study of the changes that take place in the left and right lobes of the liver at the time of birth (Emery 1952, 1953), it was noticed that more haemopoietic foci occurred in sections from one lobe of the liver than from the other. This paper reports a survey of the incidence of haemopoietic foci in the left and right physiological lobes of the liver in stillborn and live-born infants.

MATERIAL AND METHOD

Blocks of tissue of distinctive shape were taken from the left and right physiological lobes of the liver as a routine procedure at all necropsies (Emery, 1952). These tissues were processed, blocked together, sectioned and stained as one piece, enabling a direct comparison between the two distinctive-shaped pieces of liver in each microscopical preparation. Sections were stained by Masson's trichrome stain, haemalum and eosin and occasionally by Giemsa's method. In a number of instances several blocks were taken from the same lobe of the liver in order to estimate the variation in individual lobes. This was found to be remarkably small.

The sections were scanned using the same microscope and objectives. Foci of haemopoiesis were counted in ten to fifteen low-power fields from each side of the liver, the same number of fields being counted from each side in any one liver and an average field count calculated from each side of the liver. A larger number of fields were scanned in livers with fewer foci than those containing large numbers. Any section containing less than one focus in ten low-power fields was recorded as having no foci.

Only large and medium-sized aggregates of nucleated primitive blood cells were recorded as foci, small clusters of two or three cells within the liver sinuses were not counted. When haemopoiesis was very prominent and there were large confluent masses of haemopoiesis occupying more space than the liver tissue, an estimate of the number of foci had to be made. This only applied to livers containing more than fifty foci per low-power field.

The fields were scanned and counted, as far as possible, without knowing from which lobe of the liver the tissue came, but it must be realized that after some experience the right and left livers are distinguishable on a number of criteria distinct from haemopoietic foci. A consecutive series of livers was surveyed, livers only being discarded if post-mortem autolysis was marked or if gross disease such as syphilis, haemolytic disease or hepatitis was present.

The maturity of the infants was assessed upon a combination of a knowledge of the last menstrual period of the mother when this was known reliably, and on the crown-rump, crown-heel lengths of the infants using the tables of Streeter (1920) and Scammon & Calkins (1929).

RESULTS

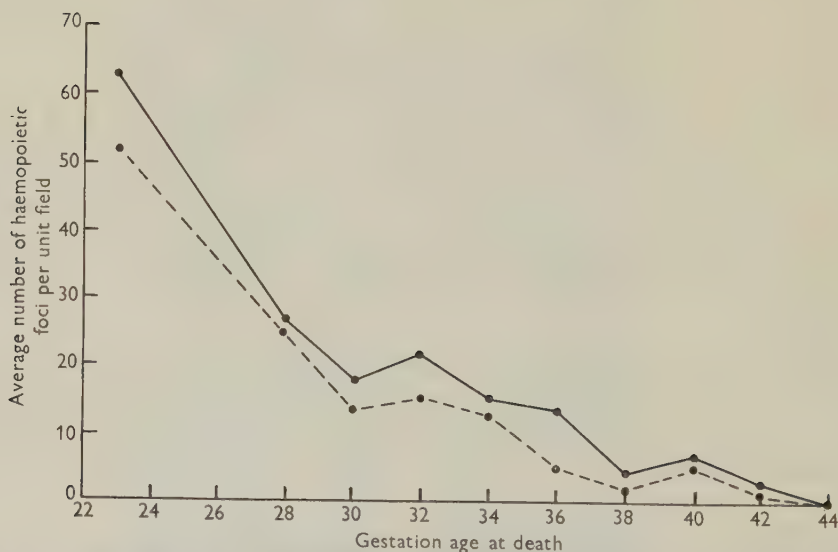
In a series of approximately 500 livers examined, 430 showed no gross pathology. Of these, 107 were from infants either stillborn or dying less than 24 hr. after birth, and 323 from children dying between the ages of 24 hr. and 6 months. The average

Table 1. *The average number of haemopoietic foci seen in the right and left lobes of the liver at different maturities*

(Stillbirths and infants surviving less than 24 hr. after birth.)

Maturity in weeks	Average number of foci per low-power field		Number of livers examined
	Right	Left	
18-27	63	52	8
28-29	27	25	8
30-31	18	13.5	9
32-33	22	15	13
34-35	15	13	3
36-37	13.5	5	5
38-39	4.5	2	8
40-41	7	4.2	38
42-43	2.5	2	12
44+	0	0	3

Total 107



Text-fig. 1. Graph showing the distribution of haemopoietic foci related to maturity in the left (interrupted line) and right (solid line) physiological lobes of the liver in a series of 107 infants dying before 24 hr. after birth.

number of foci seen in the right and left livers in different weeks of maturity is shown in Table 1, and these figures are illustrated graphically in Text-fig. 1. Owing to the relatively small number of infants in the lower maturity range, those below the age of 27 weeks have been grouped together. This is advisable also in a purely practical sense, as in livers of this range of maturity erythropoietic foci are sometimes so

numerous as to be almost confluent, and thus are much more difficult to count. Photographs of the appearance of sample livers are shown in Pl. 1, figs. 1 and 2.

The figures given report the average number of foci per field seen; the actual number of cells within the foci was not counted. There were two reasons for this, first the relative ease of counting foci as opposed to counting a large number of individual cells, and secondly that whilst the size of foci seems to vary from case to case, the size of the actual foci seemed to be relatively constant within any one liver. The aim of the study was to determine relative differences rather than absolute values.

It will be seen from Text-fig. 1 that there appears to be a constant average higher concentration of haemopoietic foci in the right than the left lobe, and that this diminishes progressively with intra-uterine age, and, in normal circumstances, hepatic haemopoiesis has ceased by 44 weeks, i.e. 4 weeks after usual full term.

There was great variation in the amount of erythropoiesis in different livers of the same maturity, thus in the eight livers examined in the age period 28–29 weeks the

Table 2. *The distribution of haemopoietic foci in 430 livers*

Maturity in weeks	Number of cases				Total
	No foci	Right greater than left	Right equal to left	Right less than left	
Children—stillbirths or surviving less than 24 hr. after birth					
18–27	0	6	2	0	8
28–29	0	5	3	0	8
30–31	0	8	1	0	9
32–33	1	9	3	0	13
34–35	0	2	0	1	3
36–37	0	4	1	0	5
38–39	0	6	2	0	8
40–41	5	20	12	1	38
42–43	1	4	7	0	12
44+	3	0	0	0	3
Total	10	64	31	2	107
Children surviving over 24 hr.	176	28	11	1	323

number of foci per low-power field varied from 2 to 100, and in the thirteen livers of maturity 32–33 weeks the number of foci varied from 0 to 100. For this reason the standard deviations of the figures in the groups are such that the differences in the means are not, within these early groups, statistically significant.

It would have been possible to analyse the number of foci seen in series of fields in the different lobes of the same liver, but this was thought to be unjustified as it would have been possible to have made any difference significant by counting an increasing number of fields.

In the livers from older children there was a smaller range of foci seen and in the largest single group, the thirty-eight infants of maturity 40–41 weeks, the standard deviation of the figures is 0.6 making the observed differences in the liver lobes statistically significant.

The actual numbers of livers showing preponderance of foci in one or other lobe is shown, related to maturity in Table 2.

Of the 107 infants dying within 24 hr. of birth, ten showed no hepatic haemopoiesis, and of these all but one were in infants of 40 or more weeks maturity. Of the remaining ninety-seven livers, two only showed more foci in the left than in the right lobes, sixty-four showing more in the right than the left lobe. No definite difference was found in thirty-one. Those livers showing no definite difference, together with the two with more foci in the left than the right liver together comprise 34% of the total, and this may be compared with sixty-four cases, or 60%, showing more in the right than the left lobes. Using the formula for standard error of a percentage $\sigma = \sqrt{(pq/n)} = 4.8$. Thus, it is unlikely that this result could have arisen by chance.

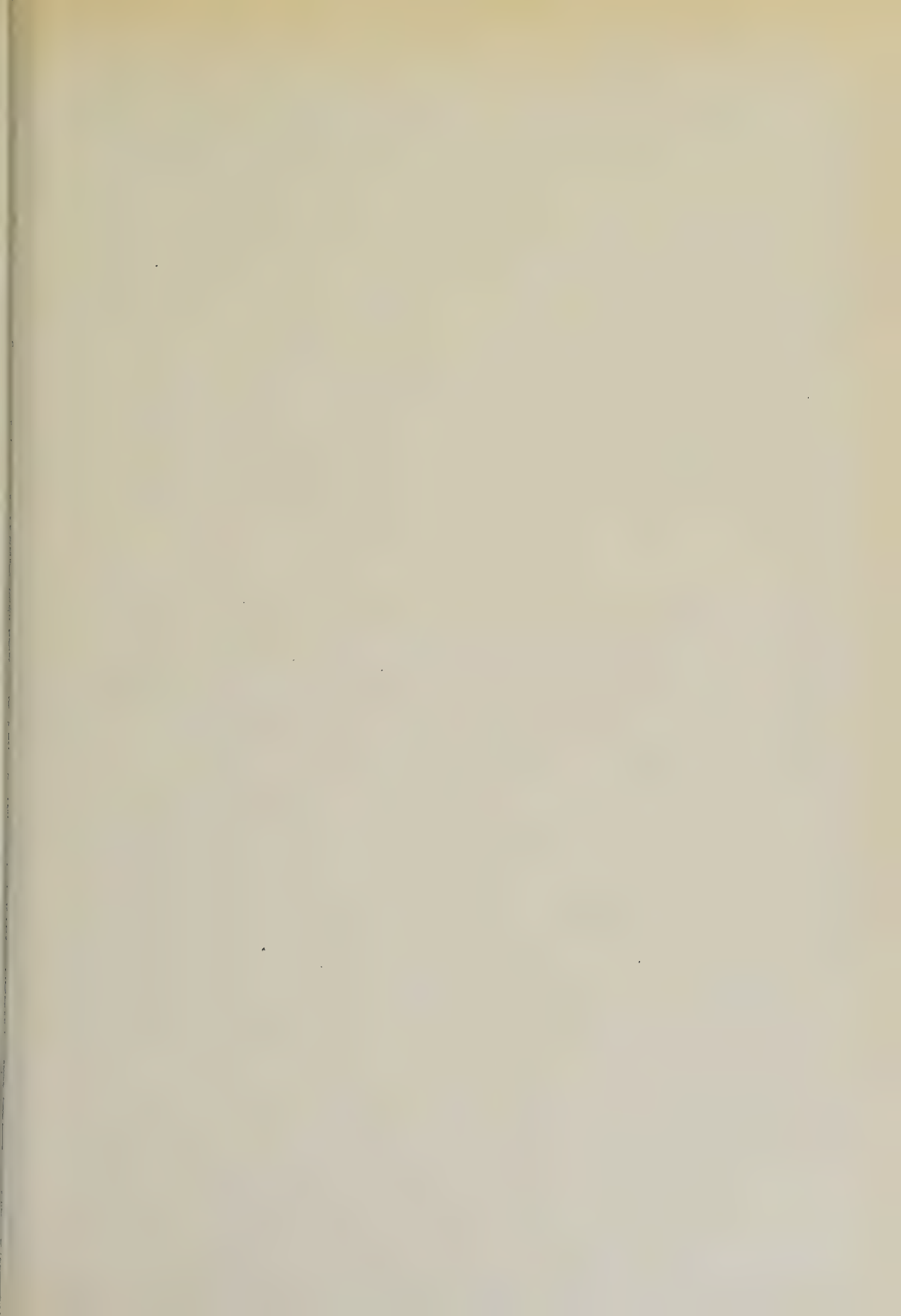
DISCUSSION

Cantle (1897-98), and later Mall (1906) in his classical studies of the liver, demonstrated that the liver consists of two distinct physiological lobes. This physiological bilaterality of the liver shows what is probably a greater difference in blood supply *in utero* than in later life, due to the peculiarities of the blood passing from the placenta through the umbilical vessels. The umbilical vein carries all the blood from the placenta to the liver. Within the liver this blood passes to the inferior vena cava by two routes—the ductus venosus and the portal vessels of the left liver. The diameter of the ductus venosus is about one-seventh that of the umbilical vein, suggesting that the bulk of the umbilical blood probably passes through the liver itself (Barron, 1944). This was demonstrated in the human newborn by radiological studies of Lind (1953). The blood from the portal vein in the foetus appears to circulate normally through the right liver. There is considerable variation in the diameter of the ductus venosus, suggesting that there is much individual variation in the relative amount of blood passing through the liver, and that any changes produced by this peculiarity of circulation would be variable.

The asymmetrical blood circulation in the liver *in utero* was considered by Gruenwald (1949) to be the cause of the relatively common fatty change in the right liver at birth; and the sudden elimination of the umbilical circulation of the left liver has been suggested as the cause of the involutionary changes in the left liver after birth (Emery, 1952), and as a factor in the production of physiological icterus (Emery, 1953).

I have been unable to find any studies of the distribution of erythropoietic foci in the liver. Gilmour (1941) examined the livers of twelve foetuses and ten newborn infants, and observed a diminution in the amount of haemopoiesis in foetuses of over 470 mm. length. This diminution in hepatic haemopoiesis with maturity was observed in the present series and appears to occur simultaneously in both lobes of the liver. It would therefore seem to be related to some factor distinct from causing the asymmetrical distribution of haemopoietic foci within the liver.

The asymmetrical distribution of the foci appears to be directly related to the difference in blood supply of the lobes *in utero*. The factor usually considered to be most prominent in the production of haemopoiesis is the oxygen tension of the blood (Grant & Root, 1952). The blood circulating in the right lobe of the foetal liver is a mixture of portal blood and blood from the hepatic artery, while that within the left liver is from the hepatic artery and from the umbilical vein. The latter is the



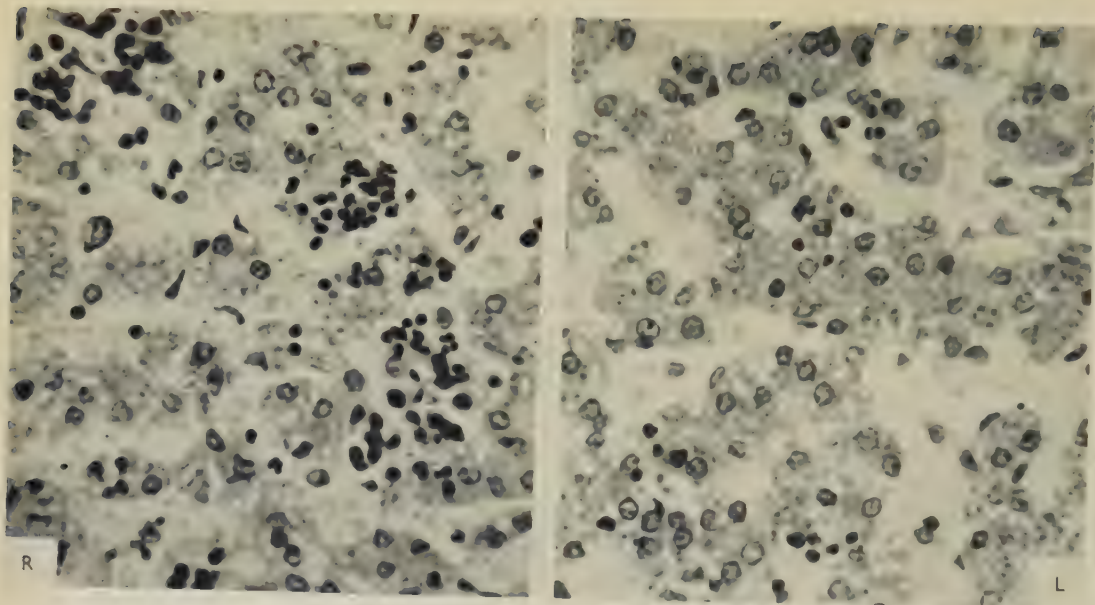


Fig. 1

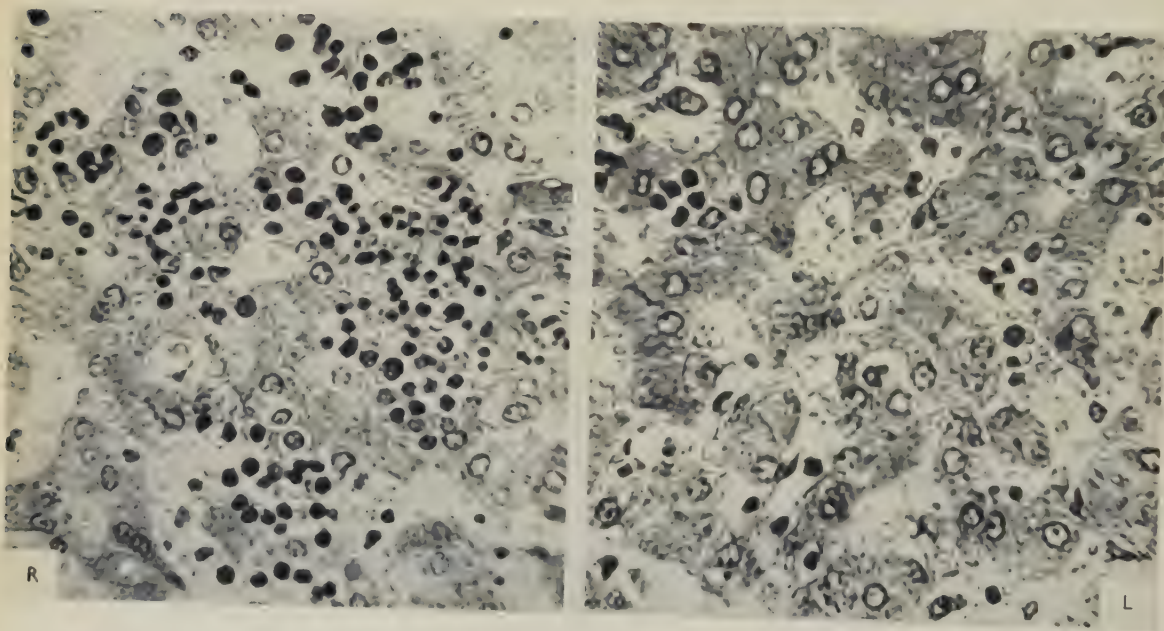


Fig. 2

EMERY—THE DISTRIBUTION OF HAEMOPOIETIC FOCI IN THE INFANTILE HUMAN LIVER

(Facing p. 297)

most highly oxygenated blood in the foetal circulation, making the oxygen tension in the left liver higher than in the right. It is obvious that substances other than oxygen will also be different in the two circulations, and may well play a part in influencing haemopoiesis.

The foetal liver thus appears to provide a natural demonstration of a difference in the constituents of the circulating blood altering the amount of erythropoiesis within a single organ.

SUMMARY

A survey of 430 livers from still and live-born infants showed that erythropoiesis was more active in the right than in the left physiological lobe of the liver. This asymmetrical blood production is ascribed to the different oxygen tension of the blood circulating through the left and right livers—blood from the umbilical vein normally passing through the left liver on its way to the inferior vena cava.

Many livers in this study were available from necropsies carried out at the City General Hospital, Sheffield, by the courtesy of Dr A. J. N. Warrack. It is also a pleasure to be able to acknowledge the advice of Dr David McCredie and Dr R. F. Wrighton, Statistician to the University of Sheffield.

REFERENCES

- BARRON, D. H. (1944). The changes in the foetal circulation at birth. *Physiol Rev.* **24**, 277–295.
- CANTLE, J. (1897–98). On a new arrangement of the right and left lobes of the liver. *J. Anat., Lond.*, **32**, 4–6.
- EMERY, J. L. (1952). Degenerative changes in the left lobe of the liver in the newborn. *Arch. Dis. Childh.* **27**, 558–561.
- EMERY, J. L. (1953). Involution of the left liver in the newborn and its relationship to physiological icterus. *Arch. Dis. Childh.* **28**, 463–465.
- GILMOUR, J. R. (1941). Normal haemopoiesis in intra-uterine and neonatal life. *J. Path. Bact.* **52**, 25–55.
- GRANT, W. C. & ROOT, W. S. (1952). Fundamental stimulus for erythropoiesis. *Physiol Rev.* **32**, 449–489.
- GRUENWALD, P. (1949). Degenerative changes in the right half of the liver resulting from intra-uterine anoxia. *Amer. J. Clin. Path.* **19**, 801–813.
- LIND, J. (1953). *Anoxia of the Newborn Infant*, p. 171. Blackwell.
- MALL, F. P. (1906). A study of the structural unit of the liver. *Amer. J. Anat.* **5**, 227–308.
- SCAMMON, R. E. & CALKINS, L. A. (1929). *Growth in the Foetal Period*. Minneapolis: University of Minnesota Press.
- STREETER, G. L. (1920). Weight, sitting height, head size, foot length and menstrual age of the human embryo. *Contr. Embryol. Carneg. Instn.* **11**, 141–170.

EXPLANATION OF PLATE

Figs. 1, 2. Sample fields from the left and right physiological lobes of two livers from stillborn infants. (Haematoxylin and eosin, $\times 400$.)

THE REPRESENTATION OF SKULL SHAPE BY CONTOUR DRAWING

BY D. I. G. BUNN AND P. TURNER

Department of Anatomy, Middlesex Hospital Medical School

Although objective data may not be completely free from subjective influence, the criteria by which skulls are assessed and compared broadly may be said to be either subjective or objective. Thus when the visual method of examination is used the assessment made is subjective, whereas if resource is made to craniometric methods involving measurements and indices a more objective judgement results. That is not to say, however, that it is necessarily the more valuable, for at the present time craniometric analysis is somewhat arbitrary, and gives little appreciation of the three-dimensional relationships of the skull parts to one another.

In examining a skull visually several methods have been used in the past. Thus the skull, or its cast, may be studied closely from various aspects, the final judgement being in the nature of a general impression. The method is wholly subjective and may appear to lack system, yet in the right hands may prove of great value.

Photographing the skull in several standard planes provides a means of systematic examination which presents certain advantages. Unfortunately this method suffers from at least two sources of error which will be discussed later.

The examination of skull outlines drawn with an instrument such as a dioptograph is of long standing, and in the comparison of two or more skulls the superimposition of the outlines obtained may provide valuable information. This method was adapted in an ingenious way by Benington (1911), who drew outlines of skulls in three planes: (1) a 'transverse vertical plane' passing through the auricular points, (2) the sagittal plane, and (3) a horizontal plane passing through the glabella; by reproducing the drawings on tissue paper the corresponding outlines of different skulls could be superimposed for purposes of comparison. Benington then combined all the corresponding outlines of a skull series into single 'type' outlines by a process of arithmetical averages. Later D'Arcy Thompson (1912) attempted a mathematical analysis of Benington's outlines, but neither the original method nor its subsequent mathematical treatment appears to have been employed by later workers.

It is suggested that the method to be described offers a new means for the visual comparison of skulls. By its use a skull may be represented on paper by a series of contours in two dimensions to give an appreciation of its true three-dimensional nature. The results obtained to date give some grounds for the belief that certain skull features, hitherto unrecognized by gross inspection, photography, the comparison of skull outlines, or the analysis of skull measurements and indices, may be revealed by this technique.

It consists essentially of drawing contours of the skull in three planes at right angles to one another, and in this way obtaining cranial 'maps'. It might properly

be considered a progressive modification of Benington's method, although the idea was developed in its entirety before either of the present authors had become aware of Benington's work.

METHOD

The skull to be examined is placed in a glass tank, supported on a bracket coupled to a universal joint which allows it to be orientated in any given plane. Water is then run into the tank, and the skull is aligned so that the water-level passes through either the Frankfurt, sagittal, or 'third' planes, the latter being at right angles to the first two, and passing through the external auditory meati.

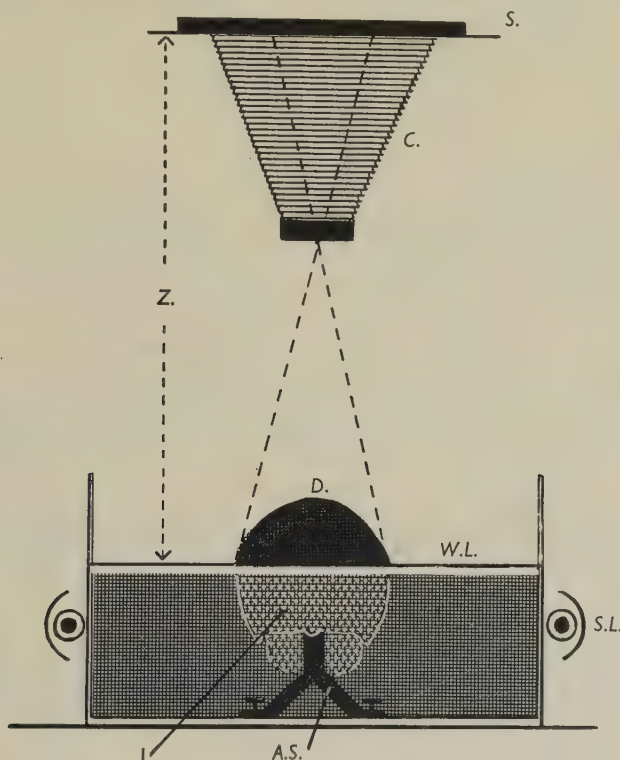


Fig. 1. Diagram of the apparatus. *A.S.*, Adjustable stand; *C.*, camera lens system; *D.*, dark island of skull surface above the water; *I.*, illuminated part of skull; *S.*, sheet of paper; *S.L.*, strip lighting; *W.L.*, water-level; *Z.*, the distance, which is kept constant, between the table and the surface of the water.

More water is then run into the tank until the skull is completely submerged. A lens system placed vertically over the skull throws the image on to a sheet of paper supported on a table above. This table is adjustable, and can be raised or lowered in order to maintain it at a fixed distance from the water-surface, as measured by a plumb line. Spirit levels are attached to the table so that it may be maintained in the horizontal plane.

The water-level is then lowered by a fixed distance, and an island of skull surface appears above the water. Strip lighting is fixed around the tank below the water-

level, its light undergoing total internal reflexion from the air-water interface. The island of skull above the water therefore appears dark in comparison with the part below, which is brightly illuminated. The water-skull surface junction can thus be clearly seen (Fig. 1).

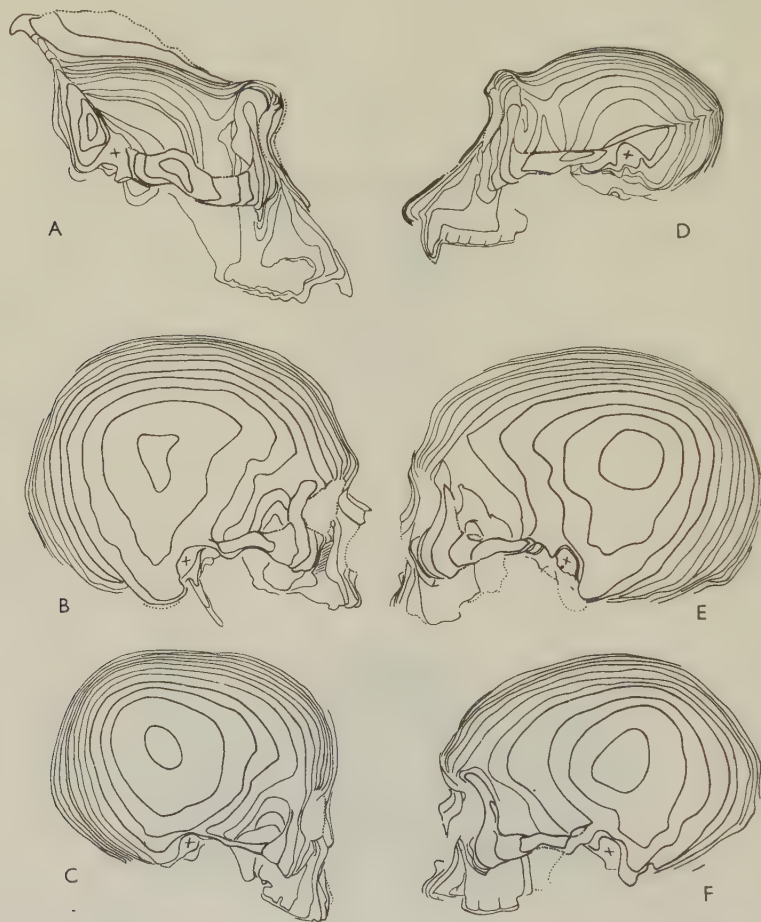


Fig. 2. Contour maps of selected skulls. A, Gorilla; B, Nordic; C, Young Mongoloid; D, Chimpanzee; E, Negroid; F, Australoid. In all cases the skulls have been drawn while held in the sagittal plane.

As the water-level is lowered by successive amounts, usually $\frac{1}{2}$ cm. in the larger skulls, but $\frac{1}{4}$ cm. in the smaller skulls such as that of the gibbon, so the lens system and table are lowered by the same distance, and outline drawings of the water-skull surface junction are made at each level with the aid of a mapping pen and inks of various colours to give the impression of depth. In this way a series of contours of the skull, or a cranial map, is obtained (Figs. 2, 3).

DISCUSSION

The technique which has been described provides a simple graphic method of representing skull shape from which a three-dimensional appreciation of form may be gained. By following the contour lines the manner in which the shape and gradient of the curved surfaces change in different parts can be readily judged. Where they lie close together the slope is steep and vice versa, and smooth surfaces which on naked eye inspection fail to arouse interest can be made to yield features previously unsuspected. In brief, the method allows a very close analysis of the progressive changes in the shape of the cranial vault not possible otherwise. A further advantage is its objective nature; maps when accurately drawn, be they ordnance survey or cranial, allow little scope for subjective interpretation.

The value of photography in anthropological investigation has been discussed by Gavan, Washburn & Lewis (1952), who pointed out certain of its shortcomings. These are in the nature of distortions, of which two forms are recognized. The first may be called the scaling error, and results from the fact that landmarks on an object which lie near the camera appear relatively larger than those at a greater distance.

The second distortion is inexactness in the image of the profile of the object being photographed, and results mainly from the masking effect produced by surface prominences. It is illustrated in Fig. 4.

Moreover, in the usual optical system employed in photography, the distance between object and camera is of necessity such that considerable convergence of the light rays takes place. The resultant distortion in outline can only be eliminated by removing the camera to a very great distance.

Since the optical system used in the present method resembles that of the camera, the same hazards are experienced. Thus convergent light rays are still used to project the image of the skull outline on to the screen, with subsequent incorrect rendering of the outline to be traced. The degree of error is small and is neither greater nor less than that inherent in the photographic method. However, in the case of the masking effect the present technique possesses a great advantage over photography; such an effect, produced by prominences lying near to the camera, can be instantly recognized by the overlapping which is produced in the contours (Fig. 5).

The scaling error in photography, i.e. inconsistency of magnification of near and far features of an object, is avoided in the contour method of representation. This is because each contour line is drawn with the skull-water junction at the same distance from the optical system, and in consequence the scale of magnification is constant, no matter how many outlines are required.

The extent to which cranial maps prepared in this way may prove of value to anthropologists remains to be seen. From our preliminary examination of those which have been prepared the impression has been gained that they may throw new light upon recognized skull features, and possibly bring new features to light. Thus in the maps showing the *norma lateralis* of the human skull a striking feature is the gradient which extends from the parietal eminence to the mastoid process. Separate descriptions of these prominences abound, but their planar relationship



A



B



C

Fig. 3

Fig. 3. Contour maps of selected human skulls. A, Australoid; B, Nordic; C, Young Mongoloid. In all cases the skulls have been drawn held in the Frankfurt plane.

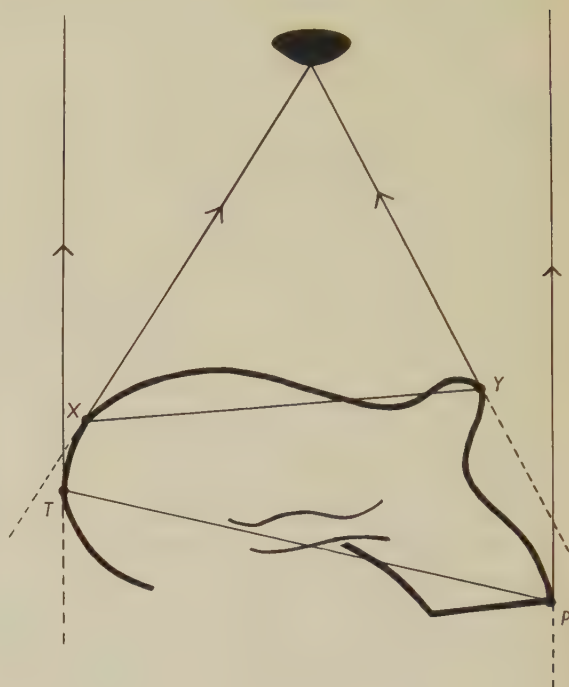


Fig. 4



Fig. 5

Fig. 5. Diagram to show how the masking effect may be recognized. Since the lines *a* and *b* are not complete, while the lines *c* and *d* are, then clearly there must be a bulge on the skull surface running from *d* to *c* which prevents visualization of the deeper lying portion of the skull.

to one another may not be possible of analysis and description without a method such as is here suggested. Its usefulness in growth studies is also a possibility.

One adaptation of the method which is quite practicable is the preparation of outlines of sections through the skull in any plane, simply by making a projection drawing from the relevant cranial map (Fig. 6). Should the accuracy of such a section be in doubt, for example in the steeper parts of the skull where the contour lines tend to bunch, it can be replaced by a direct outline obtained by orientating the skull in the water in the particular plane.

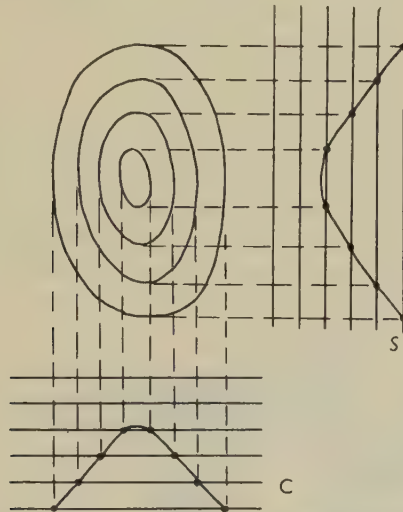


Fig. 6. Cranial map of skull-type object, norma verticalis. By projection drawing, sections cut in the sagittal and coronal planes are shown at S and C respectively.

Although the skull has been referred to throughout as the object of study, the method could be applied to any bone, provided there were satisfactory landmarks by which orientation in suitable planes could be achieved.

SUMMARY

1. A graphic method of representing the three-dimensional form of the skull is described.
2. Cranial maps, each consisting of a series of contour lines, are prepared with the skull held successively in each of three planes at right angles to one another.
3. Possible applications of the method are suggested.

We should like to thank Prof. E. W. Walls for his continued interest and advice, Dr T. J. Buchanan for his advice on optical problems, Miss E. D. Hewland for the care and skill with which she prepared the figures, and Mr C. J. L. Jarrett for much help in constructing the apparatus used.

REFERENCES

- BENINGTON, R. C. (1911). Cranial type contours. *Biometrika*, 8, nos. 1 and 2, 123-201.
 GAVAN, J. A., WASHBURN, S. L. & LEWIS, P. H. (1952). Photography: an anthropometric tool. *Amer. J. phys. Anthropol.* 10, 331-353.
 THOMPSON, D'ARCY (1912). Contour diagrams of human crania. *Nature, Lond.*, 88, 513-515.

A RAPID METHOD OF GRAPHIC RECONSTRUCTION

By C. H. BARNETT

Department of Anatomy, St Thomas's Hospital Medical School, London

By means of the technique to be described, several perspective views of an object that has been serially sectioned may be drawn to scale.

APPARATUS

The apparatus used is shown in Fig. 1. It consists of a standard microprojector—fitted with built-in illumination and a prism that throws a divergent beam vertically downwards—modified in two respects. First, the drawing-board upon which a sheet of graph-paper has been pinned is tilted at about 30 degrees to the horizontal. Secondly, a cylindrical convex lens, with a focal length of 10–15 cm., is interposed between prism and graph-paper, its power meridian parallel to the optical axis of the projector.

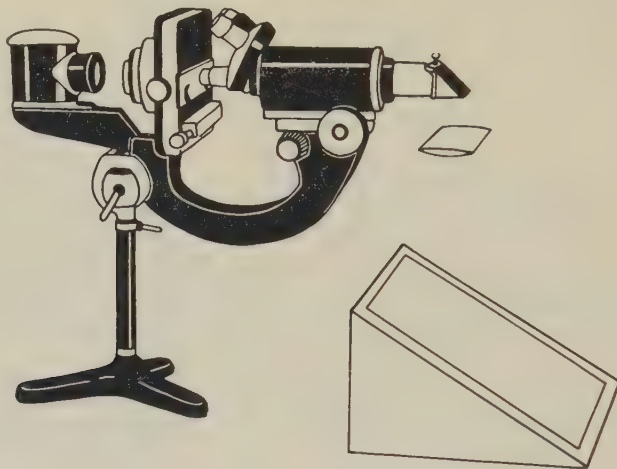


Fig. 1. The apparatus.

PRINCIPLES OF THE METHOD

The underlying principle may be explained with reference to a simple object, shown in perspective in Fig. 2, consisting of rectangular slabs each 10 units thick. When projected by means of the apparatus described, each rectangle would appear to be a trapezium. The reason for this distortion is shown in Fig. 3. The rectangle is reproduced unchanged on the left; in the middle diagram the distortion caused by tilting the drawing-board is shown; in the right-hand diagram the reduction in height has been achieved by the interposition of the cylindrical lens, which reduces the height of the image without altering its width.

It is clear that by projecting, and drawing, each section 7 units further down the

page than the preceding one, the reconstruction shown in Fig. 4 is obtained. It should be noted that each slab is made to appear only 7 units thick, the reduction from 10 units corresponding to the apparent foreshortening when an object is viewed from above at an angle of 45 degrees to its upper surface. At first sight this drawing does not resemble Fig. 2. However, had the object been divided into

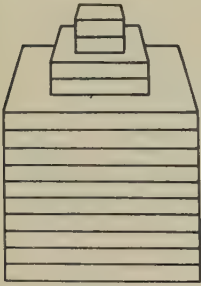


Fig. 2

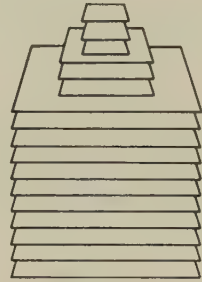


Fig. 4

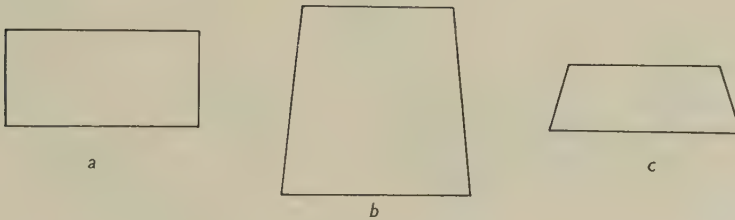


Fig. 3

Fig. 2. The object to be reconstructed.

Fig. 3. The distortion achieved by the apparatus (see text).

Fig. 4. Graphic reconstruction of the object.

microscopic sections, instead of thick slabs, the jagged edges of the reconstruction would have been much less obvious. In practice, drawings of embryos made by the present technique closely resemble perspective views of wax-plate reconstructions of the same embryos.

TECHNICAL DETAILS

The magnification of the apparatus is adjusted, using the projected image of a graduated glass slide, so that a distance of 100 μ on the slide corresponds to an exact number of small divisions on the graph-paper (say 100). The image of the scale should lie transversely across the centre of the field while the adjustment is being carried out.

The serial sections are now projected in turn. If no external guide line has been incorporated in the wax block, it is usually possible when reconstructing part of an embryo to use the neural tube as an internal guide. The first section should be orientated so that the line bisecting the image of the neural tube is projected parallel to the horizontal lines on the graph-paper. The part of the embryo that is to be reconstructed is then outlined, together with the neural tube. The second section is then projected so that the image of the neural tube lies exactly seven divisions further down the page; each section is here assumed to be 10 μ thick. The relevant

portion of the embryo is again drawn, omitting any line that would underlie the previous outline. The same procedure is followed throughout the series. The correct positioning of each section is more easily achieved if the mechanical stage on the microprojector is of the rotating type.

If required, the first section may now be projected with the line that bisects the image of the neural tube lying vertically. The whole procedure is then repeated, thereby providing a perspective view of the object from a direction at right angles to that originally chosen.

Two typical reconstructions of part of an embryo are shown in Fig. 5. If the original object changes only very gradually in form, it is unnecessary to project every section. For example, every fifth section can be taken, and each image is then projected a distance equivalent to $35\ \mu$ below the level of the preceding one.

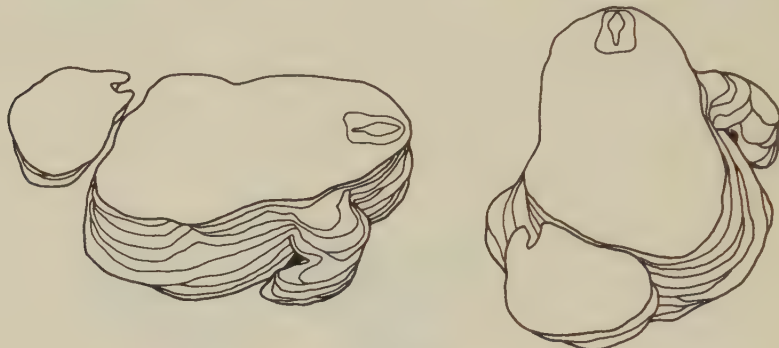


Fig. 5. Two reconstructions of part of an embryo.

Four views of any object can be drawn by turning the sections through a right angle three times. These usually suffice, but if four views from below are required in addition, it is merely necessary to reverse the order in which the sections are projected.

The exact angle of the drawing-board and the position of the cylindrical lens depend on the particular microprojector used. A simple way of setting up the apparatus for the first time is to project the image of a rectangle that has been etched on a glass slide (see Fig. 3*a*). This is focused on a horizontal drawing-board, which is then tilted until a trapezium similar to Fig 3*b* is seen. Provided the depth of focus of the apparatus is not too short, all sides of the figure are still clearly visible. The cylindrical lens, mounted on a suitable stand, is now placed below the prism and raised or lowered until the required distortion, as in Fig. 3*c*, is achieved.

When high-power objectives are being used, the depth of focus may be so short that parts of the image on the tilted drawing-board are not clearly seen. The tilt of the drawing-board may be reduced and the reconstruction made as usual if accurate proportions are not essential. For medium and low-power work the technique described is practicable in almost all circumstances where wax-plate methods can be applied.

SUMMARY

A technique is described for drawing perspective views of an object that has been serially sectioned, using a microprojector that incorporates a cylindrical convex lens.

REVIEWS

The Fossil Evidence for Human Evolution. An Introduction to the Study of Paleo-anthropology. By W. E. Le Gros Clark. (Pp. x + 181; 20 illustrations; $5\frac{1}{2} \times 8\frac{1}{2}$ in.; \$6.00 or 45s.) University of Chicago Press; Cambridge University Press. 1955.

This volume is an important addition to the literature concerned with the problem of man's ancestry. Its distinguished author requires no introduction in a journal of which he was, for a long and critical period, the Editor, and to which he has been a contributor for over thirty years. The association of British anatomy with the study of man's origin goes back to the very beginning of that subject as a science and Sir Wilfrid Le Gros Clark, in adding his present volume to this great tradition, brings to the subject great knowledge based on first-hand and extensive contact with primate and human palaeontological material. He has also the great advantage of having been able to visit a number of the important sites in Africa from which some of the most interesting fossil specimens he discusses were obtained.

The subtitle suggests that the book is an introduction. This, in the reviewer's opinion, is to pay too great a tribute to the acumen of most beginners. The general position adopted is closely argued and the first chapter, which deals with the taxonomic problems involved in the assessment of the evolutionary position of fossils will, surely, be 'hard going' for most aspiring neophytes. This chapter, however, is most important as a statement of the author's general points of view. A number of these are eminently reasonable, and attention to them will prevent some of the egregious blunders and muddled thoughts that have bedevilled the attempt to treat the history of man's origin in a scientific manner. That, as is indicated, such a treatment ought to involve the technique, the approach and the criteria used by palaeontologists, who study other animal groups would seem to be a basic requirement. In fact, as reference to many of his predecessors will soon show, human palaeontology has suffered from an extreme, not infrequently, indeed, a naughty, disregard of the elementary working rules of the palaeontologist in general. These rules and the developments of evolutionary theory in the past twenty years which have proved to be so helpful in other phylogenetic studies can, in the study of the origin of man, only be ignored at great peril to objectivity.

On two points in his introductory chapter Sir Wilfrid is forceful. One relates to the difficulties that arise from the use of colloquial terms and undefined new names. Here his reasonable readers will certainly be prepared to follow him. His criticisms, too, of the biometric approach, which are clearly and fully stated, are certainly cogent. He points out the difficulties of this approach and he lists a number of the fallacies that may arise in its application. But while that 'continuous pilgrimage towards the quantitative', which constitutes the very core of science, progresses there will always be those who measure. That they should measure to some purpose is obviously most desirable but that they measure at all is, perhaps, better than that anatomists should, because of the absence of a completely suitable three-dimensional metric—a topology that includes dimensional analysis—keep too rigorously to the narrow path of the non-metrical. But, if there is much explicit and implicit criticism in this first chapter the author engagingly disarms reaction by confessing that not a few of the criticisms apply as well to some of his own work as they do to that of others.

In the next three chapters of the volume the genera *Homo*, *Pithecanthropus* and *Australopithecus*, which are included within the family Hominidae, are discussed from the viewpoint of their taxonomic status, and criteria for their formal diagnosis are suggested. The species *Homo sapiens* is considered to include the earlier 'neanderthaloids', and it is suggested that the Krapina, Solo and Rhodesian specimens should be included within the genus, but that the available material does not allow of statement on specific position. The later, classical, 'neanderthaloids' alone are placed in the species *H. neanderthalensis*. The genus *Pithecanthropus* is divided into two species *erectus* and *pekinensis*; it is considered that the inference that this genus was ancestral to the genus *Homo*, though only a working hypothesis, is one 'which has the perfectly reasonable basis that it is consistent with the evidence so far available.'

The Australopithecinae are described in considerable detail. The term is used, as a temporary device, to include the whole collection of South African hominoid fossils to avoid confusion while the taxonomy of the various groups is still subject to uncertainty. The view is expressed, however, that the anatomical differences between the fossils found in the Transvaal are not sufficient to justify generic distinction. Sir Wilfrid considers that, as between the Pongidae and the Hominidae, the Australopithecinae must be allocated to the latter. In arriving at this conclusion use is made of the formulated provisional definition of the Hominidae which must be considered in association with the formal definition of the Pongidae. Accepting these definitions, and a strong case is made out for them, it is concluded that the Australopithecinae must be allocated to the family Hominidae. As is pointed out the only other interpretation is that the South African group of fossils represent a third radiation of the superfamily Hominoidea which shows remarkable parallelism to the Hominidae. 'But such an interpretation would be wholly gratuitous, with no supporting evidence, and it would demand a degree of evolutionary parallelism far beyond anything that has been demonstrated to have occurred in any other mammalian sequence of evolution.' While the Australopithecinae, however, are to be regarded as 'hominids in the taxonomic sense the terms "man" and "human" can hardly be applied to them, for there is no certain evidence that they possessed any of the special attributes which are commonly associated with human beings'. With this view of the *significance* (of their *importance* there can be no doubt at all) of the Australopithecinae, as is well known, there is not universal agreement. The author's clear and precise statement of his views is, therefore, most important, for no one can now have any doubts on the reasons that have led Sir Wilfrid to his conclusion that the South African fossils deserve hominid status; that, in fact, in the Villafranchian period 'there existed primitive hominids with a cranial capacity exceeding by very little that of the large anthropoid apes but with a limb structure evidently related to the development of an erect posture and gait which is so marked a characteristic of the evolutionary sequence of the Hominidae in general'.

The last chapter is concerned with the origin of the Hominidae. Some brief attention is given to the fossil history of the Hominoidea, and it is suggested that the Hominidae and the Recent Pongidae may well have had a common ancestry as late as the Early or Middle Miocene and possibly even later. Opposing trends in adaptation to posture and gait were associated with the subsequent divergence that accompanied the phylogenetic separation of the Hominidae from the Pongidae. This provisional interpretation will obviously stand or fall by the manner in which it is supported by future fossil finds. Meanwhile, the author's status and experience will ensure it a sympathetic reception.

J. D. BOYD

Blood Supply and Anatomy of the Upper Abdominal Organs. By NICHOLAS A. MICHELS, M.A., D.Sc. (£8. 0s. 0d.) London: Pitman Medical Publishing Co. Ltd. 1955.

The variability in the arrangement of the vessels in the upper abdomen has long been noted by anatomists and surgeons, and Belou, Thompson, Flint and many others have reviewed and added to the legion of reported anomalies. Now Prof. Michels has collated the available information and, as a result of many years spent in careful dissections of over 500 subjects, besides studies of other specially injected preparations of the liver and bile ducts, he has added greatly to our knowledge of upper abdominal vascular variations and their significance. The numerous illustrations are admirably clear and informative, although a monograph of this type, loaded with vascular details, is not always easy to read, and the work will find its chief use as a work of reference rather than as a text for routine study. Considering the title there are some curious but interesting interpolations on, for example, dyskinesia, hepatoscopy and religious rites, the history of the first cholecystectomy in America, the incidence of gall stones in various diseases, and so on. The actual descriptions of the upper abdominal viscera are not up to the standard of the discussions of the arteries, the veins receive relatively little attention, and the nerves are ignored apart from those innervating the sphincter of Oddi. However, these are minor criticisms of what is undoubtedly a major contribution to this field of regional angiology.

G. A. G. MITCHELL

ARTERIO-VENOUS ANASTOMOSES IN THE HUMAN EXTERNAL EAR

By M. M. L. PRICHARD AND P. M. DANIEL

*The Nuffield Institute for Medical Research, University of Oxford, and the
Departments of Neurology, Neurosurgery and Pathology,
Radcliffe Infirmary, Oxford*

Having found that arterio-venous anastomoses were present in the external ears (auricles) of a number of species of animal (Daniel & Prichard, 1956), we examined some human ears and found that large numbers of these highly specialized blood vessels, which form direct communications between arteries and veins, were present also in man. In this paper we describe the distribution and morphology of these arterio-venous anastomoses in the human ear.

METHODS

The ears were obtained at necropsy from eight subjects of both sexes ranging in age from 17 to 90, and from one newborn infant. They were excised with about 1.0 cm. of skin surrounding their line of attachment. Two specimens were injected with neoprene latex, and five with Berlin blue (2 % in distilled water). The remaining ears were not injected and were used solely for histological study. These ears were cut into horizontal blocks which were fixed either in 10 % formol-saline or in formol-mercuric-chloride (9 parts of saturated solution of mercuric chloride, 1 part of 40 % formaldehyde). After fixation the blocks were embedded in paraffin and cut at 7μ , several serial runs being made of each block. In some preparations the serial block method was used (Beesley & Daniel, 1956). Most of the sections were stained alternately with Ehrlich's haematoxylin and eosin, Weigert's elastic method, and iron haematoxylin and van Gieson; some were stained by Masson's trichrome method.

The injection masses were introduced by syringe into one or more of the main intrinsic arteries of the ear (after insertion of cannulae under the binocular dissecting microscope), the cut ends of other arteries having been located and tied off. It was found to be important to warm the ears very thoroughly in hot water before introducing the mass, so that the fat was soft and did not impede the injection.

The casts of the vessels of the ears injected with neoprene were not examined until the tissue had been completely macerated. For maceration the ear was first placed in concentrated hydrochloric acid at 37°C . for 24 hr., after which it was thoroughly washed and transferred to 10 % potassium hydroxide at 37°C . for about 48 hr. to remove the remaining fat. After the cast had been again thoroughly washed it was examined in water under the dissecting microscope.

The ears which had been injected with Berlin blue were not fixed until after the skin and much of the subcutaneous fatty tissue had been stripped (under the dissecting microscope) from both surfaces with a sharp scalpel, leaving the vascular

layer overlying the perichondrium more or less exposed on each surface of the cartilage. The specimens were then placed in 10% formol-saline. After fixation any remaining areolar tissue still covering the layer of blood vessels on the perichondrium was carefully dissected away (again under the dissecting microscope). Small areas of perichondrium, together with the overlying vascular layer, were then stripped from the cartilage, dehydrated, cleared and mounted on a slide in Canada balsam. These preparations gave a good general view of the vessels but were too thick to be satisfactory when stained. Other preparations were therefore made in which small strips of the vascular layer were gently detached from the perichondrium. These were stained with Ehrlich's haematoxylin before being cleared and mounted on a slide. We found that the stripping of the thin layers of fixed tissue was more easily done after the specimen had been immersed in absolute alcohol for a little while. These 'strip' preparations were supplemented by sections cut on the freezing microtome at various thicknesses, but the latter did not in general prove to be so useful, although they had the advantage of showing the arterio-venous anastomoses in the corium as well as those in the perichondrium.

RESULTS

Arterio-venous anastomoses were found to be present in large numbers in the human external ear. They were seen in the specimens taken from the young adults, the aged, and the newborn infant. Many of the arterio-venous anastomoses lie on or in the perichondrium on both aspects of the cartilage. Others are present in the skin, where they lie in the deeper layer of the corium, and others again are present in the subcutaneous fatty tissue. In the lobule of the ear they are also found lying deep within the fatty tissue.

The injected preparations showed that the arterio-venous anastomoses spring from vessels of varying calibre, ranging from extremely small arteries to arteries with an internal diameter of about 80–100 μ . The size of the potential lumen of different arterio-venous anastomoses is very variable, and is related to the size of the artery from which the vessel springs—the larger this artery the larger the potential lumen of the anastomosis. In some of the injected preparations nearly all the arterio-venous anastomoses showed a very small lumen, due to the constricted state of these vessels. In other similar preparations, however, the arterio-venous anastomoses appeared to be widely open, and in these the internal diameter of the majority was about 20 μ , though in many it was in the region of 40 μ , and in some of the larger arterio-venous anastomoses the internal diameter was as much as 50–60 μ . The largest arterio-venous anastomoses lie on the perichondrium, but small ones are also present at this depth.

Individual arterio-venous anastomoses also differ somewhat from one another in shape. A few are rather tortuous, but in the majority marked convolutions are absent. Some make a more or less complete S-bend (Pl. 1, fig. 3); others make only a single bend, sometimes with a wide arc (Pl. 1, fig. 1), but more often with a sharp hairpin turn (Pl. 1, figs. 2, 4, 5). Frequently, however, there is no conspicuous bend in the course of the vessel, which appears as a relatively straight connexion between an artery and a vein (Pl. 1, fig. 4).

The distinction between arteries and veins in thin strips of injected tissue, cleared but unstained (Pl. 1, fig. 1), presented no real difficulty, thanks to differences in the characteristic form and pattern of the arterial and venous systems. No valves were found in the veins of the human ear, although they are present in the veins of the ears of some animals (Daniel & Prichard, 1956). Many of such strips of injected tissue were, however, stained with haematoxylin to demonstrate the cellular features of the walls of the various vessels. The specimens illustrated in Pl. 1, figs. 2-5, were necessarily photographed at relatively low magnifications, but with the higher powers of the microscope a surprising amount of cellular detail can be seen in such material, despite the thickness of the tissue.

The walls of the arterio-venous anastomoses are not nearly so thick as are those of arterio-venous anastomoses which we have seen in some other sites, e.g. the fingers and toes of man, and they contain many fewer layers of cells (cf. Pl. 2, figs. 6, 7). The thickest wall seen in an arterio-venous anastomosis in a human ear measured about $25\ \mu$, but this was unusual, and many of the arterio-venous anastomoses had walls no thicker than about $10\ \mu$. The structure of the vessel wall, however, is essentially the same as that of arterio-venous anastomoses in other regions. The media is composed of variable numbers of epithelioid and smooth muscle cells (Pl. 2, figs. 8-13). The latter may be of the type normally seen in the wall of a small artery, or may be of modified form, having shorter and thicker nuclei. The epithelioid cells have large nuclei, which are round, oval or polyhedral in shape and often pale in colour when stained with basic dyes (Pl. 2, fig. 9). The nuclear membrane is prominent, but there is no well-defined nucleolus. The cytoplasm of these epithelioid cells stains poorly with acid dyes and the cell outline is usually ill defined. The two types of cells are disposed somewhat irregularly in the vessel wall, though the epithelioid cells tend to lie nearer or even adjacent to the lumen and the smooth muscle cells to be situated more peripherally (Pl. 2, fig. 10). Near the arterial end of an arterio-venous anastomosis smooth muscle cells predominate, but further along the vessel epithelioid cells are the more numerous. In the mid-part of the anastomosis the epithelioid cells tend to be packed closely together (Pl. 2, figs. 6, 12), sometimes in irregular groups, but towards the venous end they are often seen forming a single layer lying adjacent to the lumen (Pl. 2, fig. 13). In paraffin sections many of the arterio-venous anastomoses are rather inconspicuous structures because of their relatively small size and particularly their not very thick walls (Pl. 2, figs. 11, 13).

Except for traces of elastic tissue which may be seen near the arterial end of an arterio-venous anastomosis an internal elastic lamina is absent. Towards the venous end a layer of elastic tissue may often be seen around the outer surface of the vessel wall. Endothelial cells are present along the lumen of an arterio-venous anastomosis, but it is doubtful whether they form a continuous lining to the vessel since epithelioid cells are often seen projecting into the lumen. In paraffin sections the lumen itself is frequently extremely small and irregular in shape (Pl. 2, figs. 6 and 8-10).

DISCUSSION

So far as we are aware, the presence of arterio-venous anastomoses in the external ear of man has not hitherto been definitely established. Suequet (1862) postulated that they were present in this site, but his opinion was based on inference. Neither Berlinerblau (1875) nor Hoyer (1877) could find arterio-venous anastomoses in the human ear. Vastarini-Cresi (1903) was uncertain as to whether they existed or not. Grant & Bland (1931) were unable to find them in the one specimen they examined. The studies reported in this paper have shown that arterio-venous anastomoses are in fact present in the human ear in large numbers, but that in general they are far less conspicuous structures than they are in the ears of some species of animal (Daniel & Prichard, 1956) and in human fingers and toes. No actual counts were made, but there did not appear to be any obvious difference between the young adults and the aged, either in the number of arterio-venous anastomoses present or in their cellular features. Masson (1937), on the other hand, found that the arterio-venous anastomoses in the fingers and toes became less numerous in old age, and Popoff (1934) believed that those present in the digits of old people were atrophic.

The arterio-venous anastomoses in the human ear can be identified in paraffin sections, stained by routine methods, by the epithelioid cells and the modified smooth muscle cells which are present in variable numbers in the vessel wall. An internal elastic lamina is absent in the segment where the epithelioid cells are most numerous. In these respects the arterio-venous anastomoses in the human ear show the same structural features as are seen in arterio-venous anastomoses found in other sites, e.g. the fingers and toes of man (Popoff, 1934; Masson, 1937, and earlier papers), the external ear of the rabbit (Grant, 1930) and of many other animals (Goodall, 1955; Daniel & Prichard, 1956), the tongue of the dog, the sheep and the goat (Prichard & Daniel, 1953, 1954), and the nasal mucosa and tip of the nose of various animals (Dawes & Prichard, 1953). On the other hand, in the arterio-venous anastomoses situated in the human ear, the walls of the vessels are as a rule far less thick than are the walls of arterio-venous anastomoses present in some other sites. For this reason, in histological preparations the arterio-venous anastomoses in the human ear do not so readily attract attention as do those, for example, in the human finger or toe (Pl. 2, fig. 7), in the ears of the sheep, goat and pig (Daniel & Prichard, 1956, figs. 18–22) and in the tongue of the sheep and goat (Prichard & Daniel, 1954, figs. 8–15). It is presumably the relatively inconspicuous nature of the arterio-venous anastomoses in the human ear which has caused them to be overlooked by those who have searched for this type of vessel in this site.

It should be emphasized that many of the arterio-venous anastomoses in the human external ear are difficult to recognize in paraffin sections, partly because their walls are not very thick, but also because the cells in the walls are often less densely packed than they are in arterio-venous anastomoses in other sites. The examination of serial sections, variously stained, was of great assistance in the present work, both for tracing the course of the vessels and for determining the absence of an internal elastic lamina—a distinguishing feature of all arterio-venous anastomoses. In addition, the injected preparations proved a valuable complement to the straightforward histological material, in demonstrating the arterio-venous

anastomoses in a wholly different manner. In this particular investigation the use of the neoprene technique did not yield as useful results as it had done in previous studies (Daniel, Dawes & Prichard 1953; Prichard & Daniel, 1954; Xuereb, Prichard & Daniel, 1954*a, b*), and we learnt more about the arterio-venous anastomoses from the preparations injected with Berlin blue, particularly in regard to their relations to other vessels and their morphology.

Some of the arterio-venous anastomoses in the human ear have the tortuous course characteristic of this type of vessel in many other sites, but the majority are not greatly convoluted and some are almost straight. According to Clara (1927), Stolzenburg (1937), and Schumacher (1938), some of the arterio-venous anastomoses in the rabbit's ear are short and relatively straight, and have no epithelioid cells in their walls, but only typical or scarcely modified smooth muscle cells. As far as we have been able to determine, however, all the arterio-venous anastomoses in the human ear, whether relatively straight or convoluted, have at least a few cells in their walls which are of epithelioid type. This was also true of the arterio-venous anastomoses in the ears of many species of animal (Daniel & Prichard, 1956).

Various workers have considered the possible origin of the epithelioid cells in the walls of arterio-venous anastomoses, usually suggesting that they represent either metaplasia of fully developed smooth muscle cells or a special developmental form of primitive smooth muscle cells (for a discussion of this subject see Clara, 1939). More recently Murray & Stout (1942), as a result of tissue culture work with glomus tumours (tumours of arterio-venous anastomoses), have reported that the epithelioid cell can be identified as the pericyte of Zimmermann (1923). We have not been able to find any report of a glomus tumour in the ear, but it is probable that with increasing recognition of these tumours they will be diagnosed in this site as they have been in the fingers and elsewhere.

Whatever may be the origin of the epithelioid cells in arterio-venous anastomoses it seems clear that they must be concerned with the opening and closing of the vessel. How they accomplish this is not yet known, though Schumacher (1938) suggested that they change the size of the lumen by swelling or shrinking. He further suggested that during their shrinkage these cells might liberate a substance resembling acetylcholine. Grant (1930), who by direct visual observation studied the behaviour of arterio-venous anastomoses in the ear of the living rabbit, found that these vessels closed in response to adrenaline and to faradic stimulation of the sympathetic nerves, and that they opened in response to acetylcholine and histamine. Tischendorf (1938) came to a similar conclusion as to the effects of adrenaline and histamine, although he studied the problem by an entirely different method. The abundant nerve supply of arterio-venous anastomoses has been shown by studies of these vessels in the rabbit's ear (Grant, 1930), the digits of man (Masson, 1937), the tongue of the dog (Brown, 1937), the tongue of the sheep (Prichard & Daniel, 1954), and the sheep's ear (Daniel & Prichard, 1956). A histochemical study is being planned to determine whether the nerve endings on arterio-venous anastomoses are adrenergic or cholinergic in character.

It seems probable that an important, if not the most important, function of the arterio-venous anastomoses in the human external ear is to keep the ear warm in a cold environment, by opening up and permitting a greatly increased flow of blood

through the organ. The external ear is an extremity which is particularly liable to be affected by a cold environment because of its shape and structure. Not only is it a thin sheet of tissue but, except in the lobule, about a quarter of its thickness is taken up by cartilage, and there is thus between the two surfaces a layer which, being devoid of blood vessels, provides no warmth whatever. It is perhaps because of this relatively thick avascular layer that many arterio-venous anastomoses are present at a fairly deep level, namely on or in the perichondrium, as well as in the skin.

It is generally believed that the arterio-venous anastomoses which are present in the fingers and toes are a device for maintaining the temperature of these extremities when they are subjected to cold. That these extremities show a peculiar reaction to cold was first reported by Lewis (1930) and Grant & Bland (1931). These workers found that some minutes after the finger, for example, had been placed in crushed ice, and while it was still being cooled, the temperature of this finger ceased to fall and showed a sudden conspicuous rise. The more recent work on cold vasodilatation has lately been reviewed by Burton & Edholm (1955). Grant & Bland (1931) believed that the sudden rise of temperature during cooling was due to an increased blood flow through the part, which was brought about mainly by the opening up of the arterio-venous anastomoses in these extremities. Both Lewis (1930) and Grant & Bland (1931) noted a similar reaction to cooling in the human external ear but, failing to find arterio-venous anastomoses in this site, Grant & Bland attributed the response here to another mechanism. The present work makes it clear that the reaction to cooling shown by the ear can in fact also be attributed to the opening up of arterio-venous anastomoses. The theory that arterio-venous anastomoses play a major part in the phenomenon of cold vasodilatation gains support from Grant's (1930) visual observations on the arterio-venous anastomoses in the ear of the living rabbit, for he actually saw these vessels opening up when the ear had been cooled for a while. This work was confirmed and extended by Grant, Bland & Camp (1932). However, the arterio-venous anastomoses in the rabbit's ear also open up when the body of the animal is heated (Grant, 1930; Clark & Clark, 1934), which suggests that in this animal the arterio-venous anastomoses in the ear also serve as a means of lowering body temperature. For various reasons, including the small size of the ear in proportion to the size of the body, it seems unlikely that the arterio-venous anastomoses in the human ear play more than a minor part in lowering the body temperature.

In the rabbit's ear the arterio-venous anastomoses were seen by Grant (1930) and Clark & Clark (1934) to show spontaneous rhythmic changes in calibre. It seems likely that it is some such phasic activity of the arterio-venous anastomoses which gives rise to the 'hunting' phenomenon seen in cold vasodilatation, a phenomenon which was observed by Lewis (1930) in man in various peripheral sites, including the ear, and which has been studied more recently by many workers (see Burton & Edholm, 1955).

All the available evidence, direct or indirect, points to the fact that arterio-venous anastomoses are highly active blood vessels, which are capable both of complete closure and of wide dilatation, and that according to circumstances they fluctuate in calibre between these two extremes. Although the purposes which they serve and the mechanism by which they are controlled are not yet fully known there can be

no doubt that arterio-venous anastomoses are vessels of profound significance. In sites such as the human ears and digits their numbers are so great and their potential calibres so large that the amount of blood flowing through the part must be enormously different according to whether the arterio-venous anastomoses are open or closed. Consequently, when observations are made on the circulation through territories where arterio-venous anastomoses are known to be present it is essential to consider the contribution made by the activity of these vessels.

SUMMARY

Arterio-venous anastomoses are present in large numbers in the human external ear, both in and beneath the skin and on and in the perichondrium. They are not so tortuous as those present in the fingers and toes of man, and their walls are not so thick, containing fewer layers of cells. The actual cells, however, in the vessel wall are of a similar kind, consisting of epithelioid cells and smooth muscle cells, mainly of modified type, which are present in variable numbers and are irregularly disposed. An internal elastic lamina is absent.

We are grateful to Miss P. A. Alderton for help with the histological preparations.

REFERENCES

- BEESLEY, R. A. & DANIEL, P. M. (1956). A simple method for preparing serial blocks of tissue. *J. clin. Path.* **9**. (In the Press.)
- BERLINERBLAU, F. (1875). Ueber den directen Uebergang von Arterien in Venen. *Arch. Anat. Physiol. (Lpz.)*, pp. 177-188.
- BROWN, M. E. (1937). The occurrence of arterio-venous anastomoses in the tongue of the dog. *Anat. Rec.* **69**, 287-297.
- BURTON, A. C. & EDHOLM, O. G. (1955). *Man in a Cold Environment*. London: Edward Arnold (Publishers) Ltd.
- CLARA, M. (1927). Die arterio-venösen Anastomosen der Vögel und Säugetiere. *Z. ges. Anat. 3. Ergebn. Anat. EntwGesch.* **27**, 246-301.
- CLARA, M. (1939). *Die arterio-venösen Anastomosen*. Leipzig: J. A. Barth Verlag.
- CLARK, E. R. & CLARK, E. L. (1934). Observations on living arterio-venous anastomoses as seen in transparent chambers introduced into the rabbit's ear. *Amer. J. Anat.* **54**, 229-286.
- DANIEL, P. M., DAWES, J. D. K. & PRICHARD, M. M. L. (1953). Studies of the carotid rete and its associated arteries. *Phil. Trans. B*, **237**, 173-208.
- DANIEL, P. M. & PRICHARD, M. M. L. (1956). Arterio-venous anastomoses in the external ear. *Quart. J. exp. Physiol.* **41**, 107-123.
- DAWES, J. D. K. & PRICHARD, M. M. L. (1953). Studies of the vascular arrangements of the nose. *J. Anat., Lond.*, **87**, 311-322.
- GOODALL, A. M. (1955). Arterio-venous anastomoses in the skin of the head and ears of the calf. *J. Anat., Lond.*, **89**, 100-105.
- GRANT, R. T. (1930). Observations on direct communications between arteries and veins in the rabbit's ear. *Heart*, **15**, 281-303.
- GRANT, R. T. & BLAND, E. F. (1931). Observations on arteriovenous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. *Heart*, **15**, 385-411.
- GRANT, R. T., BLAND, E. F. & CAMP, P. D. (1932). Observations on the vessels and nerves of the rabbit's ear with special reference to the reaction to cold. *Heart*, **16**, 69-101.
- HOYER, H. (1877). Ueber unmittelbare Einmündung kleinster Arterien in Gefäßäste venösen Charakters. *Arch. mikr. Anat.* **13**, 603-644.
- LEWIS, T. (1930). Observations upon the reactions of the vessels of the human skin to cold. *Heart*, **15**, 177-208.

- MASSON, P. (1937). *Les glomus neuro-vasculaires*. Paris: Hermann et Cie.
- MURRAY, M. R. & STOUT, A. P. (1942). The glomus tumor. Investigation of its distribution and behavior, and the identity of its 'epithelioid' cell. *Amer. J. Path.* **18**, 183-203.
- POPOFF, N. W. (1934). The digital vascular system. *Arch. Path. (Lab. Med.)*, **18**, 295-330.
- PRICHARD, M. M. L. & DANIEL, P. M. (1953). Arterio-venous anastomoses in the tongue of the dog. *J. Anat., Lond.*, **87**, 66-74.
- PRICHARD, M. M. L. & DANIEL, P. M. (1954). Arterio-venous anastomoses in the tongue of the sheep and the goat. *Amer. J. Anat.* **95**, 203-225.
- SCHUMACHER, S. (1938). Über die Bedeutung der arteriovenösen Anastomosen und der epitheloiden Muskelzellen (Quellzellen). *Z. mikr.-anat. Forsch.* **43**, 107-130.
- STOLZENBURG, H. J. (1937). Experimentelle Untersuchungen über das Verhalten der arterio-venösen Anastomosen. *Z. mikr.-anat. Forsch.* **41**, 348-358.
- SUCQUET, J. P. (1862). *D'une circulation dérivative dans les membres et dans la tête chez l'homme*. Paris: Adrien Delahaye.
- TISCHENDORF, F. (1938). Experimentelle Untersuchungen zur Histo-Biologie der arterio-venösen Anastomosen. *Z. mikr.-anat. Forsch.* **43**, 153-178.
- VASTARINI-CRESI, G. (1903). *Le anastomosi artero-venose nell'uomo e nei mammiferi. Studio anatomo-istologico*. Naples: F. Sangiovanni.
- XUEREB, G. P., PRICHARD, M. M. L. & DANIEL, P. M. (1954*a*). The arterial supply and venous drainage of the human hypophysis cerebri. *Quart. J. exp. Physiol.* **39**, 199-217.
- XUEREB, G. P., PRICHARD, M. M. L. & DANIEL, P. M. (1954*b*). The hypophysial portal system of vessels in man. *Quart. J. exp. Physiol.* **39**, 219-230.
- ZIMMERMANN, K. W. (1923). Der feinere Bau der Blutcapillaren. *Z. ges. Anat. 1. Z. Anat. Entw.-Gesch.* **68**, 29-109.

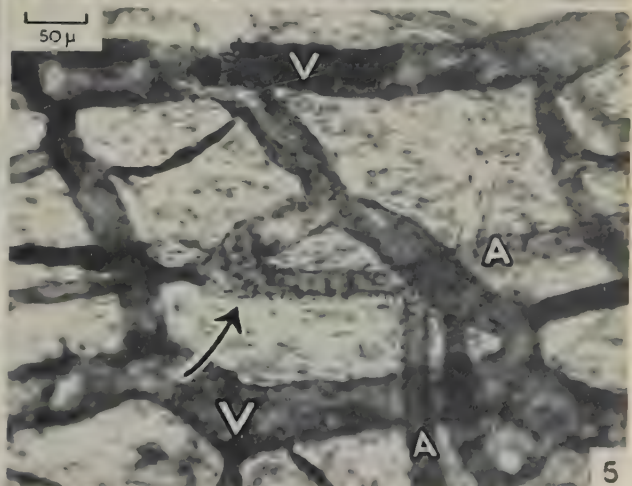
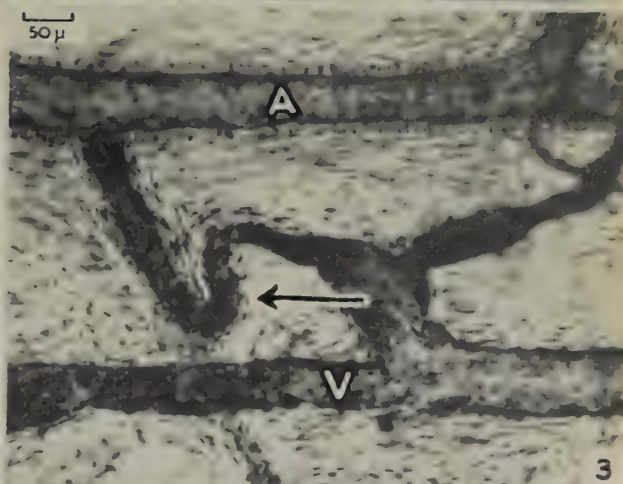
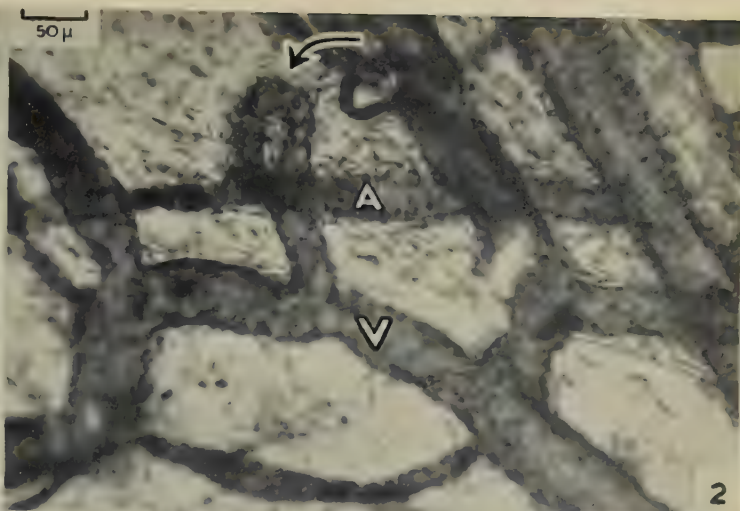
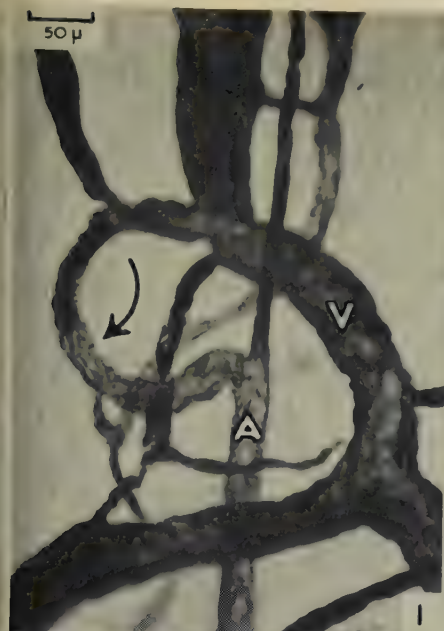
EXPLANATION OF PLATES

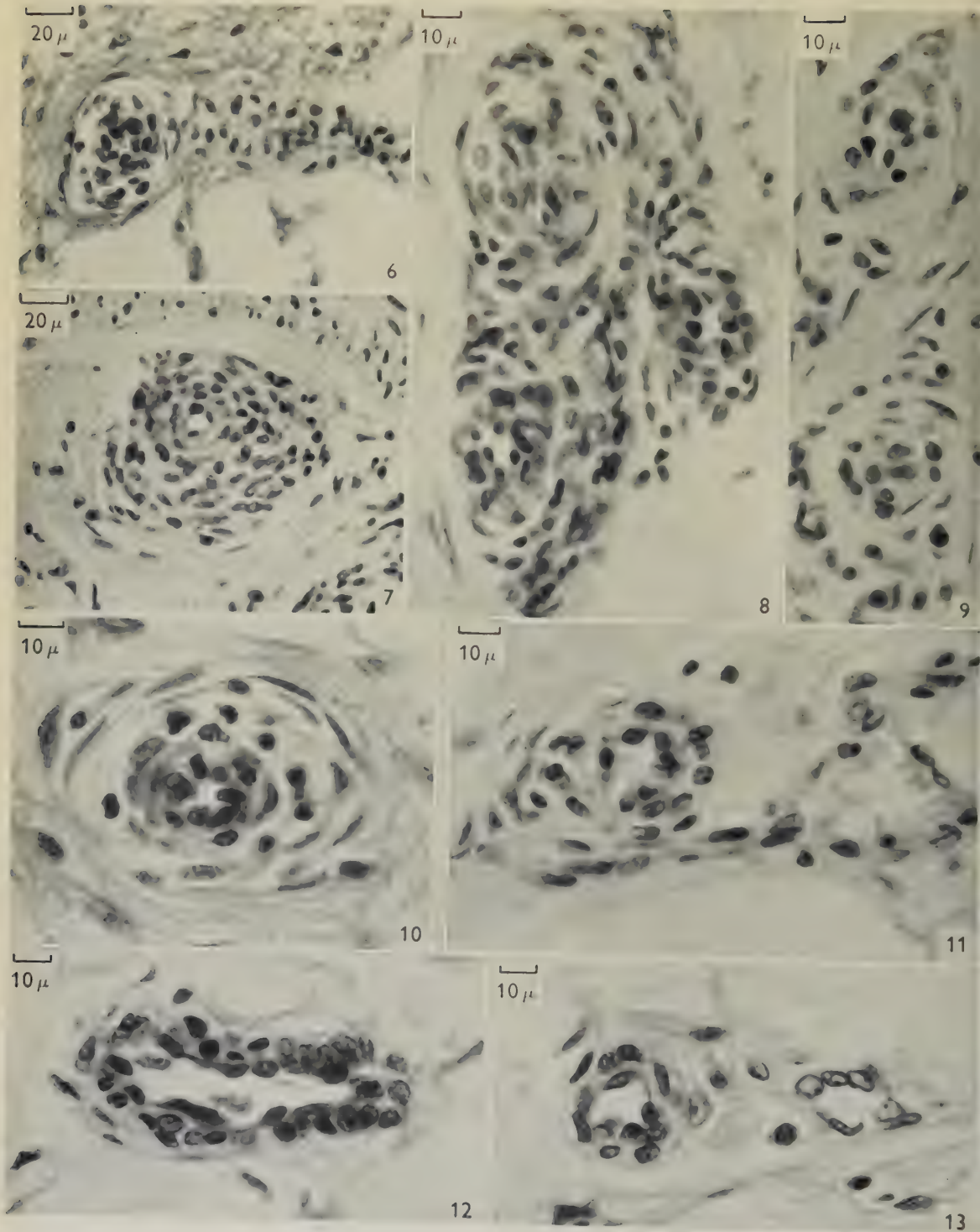
PLATE 1

Figs. 1-5. Preparations of the human external ear injected with Berlin blue. These thin pieces of tissue, stripped from the cartilage, show arterio-venous anastomoses (at arrows) in the vascular layer overlying the perichondrium. Note the large size of these channels which form direct connexions between arteries (A) and veins (V), and their relatively simple form. The examples illustrated show the typical shapes of the majority of the arterio-venous anastomoses found in the human ear, although more convoluted ones are also present. Note in figs. 1, 2 and 5 a single bend, in fig. 3 an S-bend, and in fig. 4 a long hair-pin bend (lower left arrow) and an almost straight vessel (upper arrow). The walls of these arterio-venous anastomoses are much less thick and cellular than are those of the arterio-venous anastomoses present in some other sites, e.g. in the human digits (see Pl. 2), and in the ears of some animals (Daniel & Prichard, 1956). Fig. 1 shows an injected preparation cleared and mounted unstained. Figs. 2-5 show similar preparations stained with haematoxylin before being cleared.

PLATE 2

- Fig. 6. Arterio-venous anastomosis in the corium of a human external ear, cut transversely (left), with its afferent artery approaching it from the right. Note the thick wall of the arterio-venous anastomosis, containing many epithelioid cells packed closely together, and the very small, irregular lumen. This is an example of one of the more conspicuous arterio-venous anastomoses found in the human ear, but even so it is not so prominent a structure as are the arterio-venous anastomoses present in the fingers and toes of man (see fig. 7). Iron haematoxylin and Van Gieson.
- Fig. 7. Arterio-venous anastomosis in the pad of a human toe, cut transversely. Compare this figure with fig. 6 and the other illustrations on this Plate, and note the much thicker wall of this arterio-venous anastomosis and its more numerous layers of cells. The actual cells, however, which consist of many epithelioid and some modified smooth muscle cells, are similar to those which form the walls of arterio-venous anastomoses in the human ear. Haematoxylin and eosin.





M. M. L. PRICHARD AND P. M. DANIEL —ARTERIO-VEINOUS ANASTOMOSES IN THE HUMAN EAR

- Fig. 8. Three segments of a tortuous arterio-venous anastomosis lying within the perichondrium of the human external ear. Note the great cellularity of the wall of this relatively complex vessel. Haematoxylin and eosin.
- Fig. 9. Two arterio-venous anastomoses in the corium of the human external ear. In the lower of these two vessels the pallid nuclei of several epithelioid cells are seen; its lumen is closed. Haematoxylin and eosin.
- Fig. 10. Arterio-venous anastomosis in the corium of a human external ear cut transversely. Epithelioid and modified smooth muscle cells are present in the wall of this vessel. The lumen is small and cells project into it. Haematoxylin and eosin.
- Fig. 11. Arterio-venous anastomosis (left) and small vein (right) lying on the perichondrium of a human external ear. This example is typical of many arterio-venous anastomoses in the human ear, which have only a relatively small number of epithelioid cells in their walls and are consequently not very conspicuous. Haematoxylin and eosin.
- Fig. 12. Arterio-venous anastomosis, situated in the centre of the fatty tissue of the lobule of a human external ear, cut mainly longitudinally. Note the many cells, mainly epithelioid, packed closely together in the not very thick vessel wall. Haematoxylin and eosin.
- Fig. 13. Two segments of an arterio-venous anastomosis lying just beneath the corium of a human external ear. The segment on the left is typical of the mid-portion of a small arterio-venous anastomosis in the human ear, and shows epithelioid and some modified smooth muscle cells irregularly disposed in the vessel wall. On the right the anastomosis is approaching its venous end and shows epithelioid cells, with very pale nuclei and prominent nuclear membranes, bordering the lumen. Haematoxylin and eosin.

OBSERVATIONS ON THE BLOOD SUPPLY OF THE RABBIT'S EAR AND ON THE EXPERIMENTAL NEW- FORMATION OF ARTERIO-VENOUS ANASTOMOSES

BY BRUNO ROSSATTI*

Department of Anatomy, University of Cambridge

INTRODUCTION

Since Hoyer (1877) published his classical paper on arterio-venous anastomoses (a.v.as.), pointing out that these connexions are particularly numerous in the rabbit's ear, a number of structural and experimental observations have appeared on the subject. Vastarini-Cresi (1902), Schumacher (1907, 1938), Clara (1927), Boyd (1939) and, recently, Staubesand & Genschow (1952) have made contributions to our knowledge of the structure of these vessels, and have paid particular attention to the intermediate portion of the a.v.as., which sometimes presents a thick wall with epithelioid modification of the media. Experimental investigations designed to clarify the functional significance of the epithelioid cells of this intermediate portion have been reported by Stolzenburg (1937) and by Tischendorf (1938). The behaviour of a.v.as. in physiological and experimental conditions was first studied in living animals by Grant (1930). His data were enriched later by Clark & Clark (1934), who studied the a.v.as. in transparent chambers inserted into rabbit's ears. Although the a.v.as. have been the object of such detailed studies, the general arterial and venous arrangement of the rabbit's ear has not attracted much attention, and information is still meagre on the distribution of these arterio-venous shunts and on their relationships with the arterial and venous systems. Their relative frequency at different ages and possible modification during ageing have also received little attention. It is with these aspects of a.v.as. that the present contribution is largely concerned. Observations are also recorded on regeneration of a.v.as. after the normal vascular arrangement has been modified and interrupted by the removal of a small portion of the marginal border of the ear where these shunts are always present in large numbers.

MATERIAL AND METHODS

For this investigation thirty-five rabbits were used, twenty of which were adults, seven of different ages between 2 and 7 weeks, and the remainder newborn. Most of the animals were injected with neoprene latex. The injection mass was introduced into the common carotid artery at pressures varying between 110 and 300 mm. Hg, by means of a small glass cannula connected with the usual pressure apparatus. In some animals both the posterior auricular artery and vein were injected directly from the dorsal surface of the ear at a pressure of 80–90 mm. of line Hg. The corrosion of the specimens previously injected with latex was done with a

* British Council Scholar from Anatomy Department, University of Ferrara.

50 % solution of hydrochloric acid at a temperature of 37° C. The casts were kept in water and examined, and dissected under a binocular microscope. Previous perfusion of the vascular bed of the ear with a saline solution at 37° C. was not found to give any appreciable advantage. Following the same technique, indian ink injections were also made and, after fixation in 10 % formalin, the ears were dehydrated and cleared in xylol or benzene. After dissection from the cartilage, the two skin surfaces were mounted in balsam or embedded in paraffin wax and sectioned at 100–150 μ . Small portions of different areas of the ear were fixed in formol-saline, Susa, Bouin, and Zenker fluids, and were sectioned serially. The following staining methods were used for the study of the structure of the vessels: Masson, Mallory-Azan, haematoxylin-eosin, and Unna-Taenzer for elastic tissue.

The recognition of the arterial or venous nature of the vessels in the cast preparations did not present difficulties; it is readily possible to follow the vessels to their origin from the larger branches, and in the casts the veins appear with a flat, thick set, rough surface, while the arteries present a cylindrical, smooth surface.

For the experimental part of this work, the resection of small portions of the ear border (40 by 3 mm.) was done after previous depilation and disinfection of the ear. The margin of the resected portion was covered with an 8 % solution of celloidin. After 36–40 hr. the celloidin was removed by means of an alcohol-ether solution. The animals were killed after different periods of time (3 days, 2, 3, 4 and 8 weeks) and the techniques described above were employed to assess the results of these experiments.

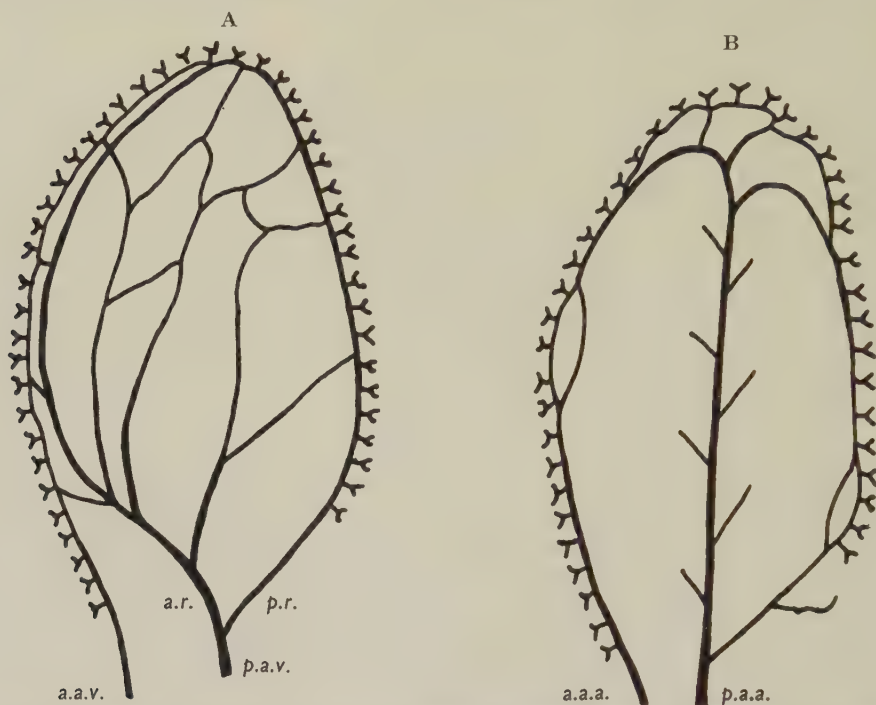
THE ARTERIES

The rabbit's ear possesses two main arteries: the anterior auricular artery and the posterior auricular artery (Text-fig. 1). The first runs on the anterior border of the ear, accompanied by the corresponding vein, and after a short course, about half the length of the border, divides into two branches which inosculate with two corresponding terminal ramifications of the anterior ramus of the posterior auricular artery to form an annular arterial anastomosis. The larger posterior auricular artery runs along the medial surface of the ear, from its root to its tip, accompanied for a short distance by the posterior auricular vein. Near the ear tip (about 1–2 cm.) it presents a triple division, the branches of which are respectively the medial, posterior and anterior rami, following the terminology of Krause (1884). Each of the last two named vessels loops towards the border corresponding to its name and runs, recurrently, in a proximal direction. The anterior ramus inosculates with the anterior auricular artery in the manner above-mentioned, the posterior ramus shows an analogous anastomosis with the largest of the branches arising, with monopodial ramification, from the posterior auricular artery itself, during its course in the central region of the ear. The medial ramus of the posterior auricular artery is distributed mainly to the tip of the ear and possesses analogous anastomoses, which are of smaller calibre. This is the normal method of ramification of the posterior auricular artery in the apical portion of the ear. Sometimes, however, this artery gives off only two recurrent rami, from the largest of which one or two small branches for the ear tip originate; the medial ramus is then often replaced by ramifications of the anterior ramus. Other arterial annular anastomoses are

also found scattered in the central regions: they are formed by smaller vessels. The arteries parallel to the border of the ear give off numerous collateral branches, which reach the edge of the ear after dichotomous division (Text-fig. 1). From these branches many a.v.as. always arise.

The blood supply of the ventral surface of the ear is provided by arteries which perforate the cartilage and are radially distributed to the surrounding tissue. On this surface arterio-venous shunts are also present.

On microscopic examination, the largest branches of the ear do not show any particular structural arrangement: the three tunicae, which are normally found in



Text-fig. 1. Schematic drawing to show the arterial (B) and venous (A) patterns of the rabbit's ear. *a.a.v.*, anterior auricular vein; *p.a.v.*, posterior auricular vein; *a.r.*, anterior ramus; *p.r.*, posterior ramus; *a.a.a.*, anterior auricular artery; *p.a.a.*, posterior auricular artery.

arteries of this size, are clearly recognizable. Occasionally in the smaller ramifications a structural modification of the wall was observed; this consisted of thick longitudinal layer of smooth muscle fibres, disposed between the intima and the normal circular musculature of the media. These longitudinal bundles of muscle fibres are situated round the whole perimeter of the vessel. Inside this muscular formation there are two or three layers of epithelioid cells, under the endothelium, so that the artery presents a fairly thick wall and a proportionally narrow lumen. Similar histological modifications have been found in a small artery of the calibre of 180μ , immediately before the point of bifurcation. The two branches arising from this artery did not possess a corresponding modification of the media. The epithelioid modification of the media has also been observed in many arterioles.

THE VEINS

The venous system also possesses an annular anastomotic disposition; there are two main trunks: an anterior auricular vein and a larger posterior auricular vein (Text-fig. 1). The former runs along the corresponding border, accompanied by the anterior auricular artery and anastomoses with ramifications of the posterior auricular vein along the length of the border. The posterior auricular vein is formed by the confluence of two branches (the posterior and anterior rami) near the root of the ear and runs, for a short distance, in the medial region of the ear, accompanied by the posterior auricular artery. The smaller posterior ramus runs in the corresponding border and at the ear tip inosculates with veins coming from ramifications of the second mentioned larger branch (anterior ramus) of the posterior auricular vein. The posterior ramus gives off three further branches which are directed mainly to the medial region of the ear, forming mutual annular anastomoses.

The ramification of the posterior ramus running on the posterior border presents a larger calibre, a straighter course and its distance from the edge is more regular in comparison with its fellow of the contralateral side. Numerous veins perforating the cartilage drain the ventral surface of the ear and are connected with larger veins of the dorsal surface.

The veins running parallel to the border give off numerous branches which reach the edge of the ear after dichotomous division like the corresponding arteries. Thus the venous system has numerous annular anastomoses in which the blood circulation is regionally subdivided; in all these regions the veins are often directly connected with arterial vessels.

No special remarks on the structure of the veins need to be made. In microscopic sections they show a thin wall formed by the endothelium and by collagenous fibres mixed with sparse elastic tissue. Staubesand & Genschow (1952) emphasized the number of valves, near the point of inosculation of an artery into a vein, in both small and large veins. There is evidence of the presence of these valves in my neoprene latex specimens, for the casts of the veins often show an annular narrowing at their origin from the main branches and, not infrequently, along their course.

THE ARTERIO-VENOUS ANASTOMOSES (a.v.as.)

In the rabbit's ear both surfaces possess a large number of a.v.as. The dorsal convex surface, however, is more richly provided with them, probably on account of the thickness of the subcutaneous tissue and of the presence there of all the main vascular branches. The a.v.as. are universally distributed on this surface of the ear, but on the edge they are always present in larger numbers. Any portion of the border shows many of these anastomotic channels. Therefore their number increases from the central region of the ear to the border. I find an average of 20–30 a.v.as. per cm.² on the marginal side as compared with 15–20 per cm.² on the lateral and medial surfaces. These figures agree with those of 25–50 per 1.6 cm.² given by Clark & Clark (1934).

As far as I could observe from the examination of the cast specimens, the shape, the course, the dimension and the length of the a.v.as. are closely related with the mutual topographical relationships between the arteries and the veins connected

by them. Practically every anastomosis has its individual characteristics. If an artery is crossing a vein, the a.v.a. will likely be at the point of contact of the two vessels and the anastomosis will be short, convoluted and branching. If the artery runs more or less parallel to the vein, the a.v.a. often will be longer, less tortuous and, again, ramifying (Pl. 1, figs. 3, 5). Arteries and veins with parallel course and connected by a simple anastomotic channel ('Bridge anastomoses' of Staubesand & Genschow) are less frequent. Indeed, there are usually several ramifications of an artery emptying into veins or directly passing into veins. If the a.v.as. are between arterial and venous terminal branches, as along the border, the vessels are connected in the form of termino-terminal inosculation; the a.v.as. there show a very large lumen which exceeds several times the average diameter of the other a.v.as. situated in other areas of the ear (Text-fig. 2; Pl. 1, fig. 4). All these anastomoses originate mostly from small arterial branches, seldom from main arteries. The veins in which they are emptying are usually of a calibre twice as large as those of the arteries. The narrowing of the anastomotic segment (intermediate portion) does not often appear in the cast preparations. The average diameter of the cast of the lumen of this segment is about four, or more, times that of the capillary vessels. Measurements show that this diameter is between 70 and 250 μ ; while the diameter of the capillary vessels is about 16 μ instead of the normal 6 or 7 μ . So the real diameter of the lumina of these a.v.as. should vary between 30 and 100 μ . It should be pointed out that in the border of the rabbit's ear the a.v.as. possess a characteristic appearance, due to their very large lumina and to the form of the connexion itself (termino-terminal), which offers a wide and rapid passage to a considerable quantity of blood proceeding from the arterial to the venous system.

After the extensive and detailed studies on the structure of the a.v.as. of Hoyer (1877), Vastarini-Cresi (1902), Schumacher (1907, 1938), Clara (1927) and Staubesand & Genschow (1952), it is not easy to add anything new. Schumacher's opinion that there are two different types of a.v.as. (one with epithelioid modification of the media, the other with normal arterial structure) could, however, be confirmed: the latter type seemed to be more frequent.

Particular attention was paid to the structure of the a.v.as. situated in the border of the ear. In microscopic sections, cut in different planes, they do not usually have an epithelioid modification of the media; mostly they present a wall formed by the endothelium and by a thin layer of muscle fibres, surrounded by the collagenous fibres of the adventitia. It is only the arterioles of smaller calibre which usually show epithelioid differentiation of the wall. The stratification of the epithelioid cells is normally limited to one or two layers; I seldom observed three layers of these cells, and then only for a very short distance. I did not find the multiple stratification which appears in other a.v.as., such as those of the glomus coccygeum (Schumacher, 1907, 1938), of the tongue of the dog and other mammals (Prichard & Daniel, 1953) or of the human nasal septum (Rossatti, 1954). The course of these vessels is not particularly tortuous or with glomerular disposition. In any case surrounding the layers of epithelioid cells there are smooth muscle fibres, mainly with circular disposition.

The observations directed to finding if these shunts are present in newborn and young animals led to the conclusion that the a.v.as. are already present with the

shape, disposition and relationships of the adult in animals 2 weeks old. Although the difficulties existing for neoprene injections in newborn rabbits forced me to resort to indian ink injections, which are obviously less efficient, I could nevertheless observe some rare arterio-venous shunts, even in animals at this stage of life.



Text-fig. 2. Arterio-venous anastomoses of the ear border (solid black) between arterial (white) and venous (stippled) dichotomic branches directed to the edge. $\times 22$.

THE EXPERIMENTAL FORMATION OF NEW ARTERIO-VENOUS ANASTOMOSES

In the preceding description it has been explained that the arteries and veins running along the border of the ear give off numerous short branches which are directed perpendicularly to the edge. These vessels possess many terminal and collateral branches which, as has been stated, are often connected by arterio-venous

shunts (Text-fig. 2; Pl. 1, fig. 4). Resections of small portions of the border were performed to remove such arterial and venous branches, and with them the existing a.v.as. After resection only short portions of the branches springing from the vessels running parallel to the border remained.

The first observations were made 72 hr. after the operation in order to determine the extent of the resection itself and the condition of the blood circulation in that area. At this stage a dilatation of the capillary nets was observed, and very rare connexions between arteries and veins with lumina larger than those of the capillary vessels.

Two weeks after operation, the vascular arrangement appeared completely modified as compared with that observed after 3 days. In cast preparations the new margin of the region of resection showed a large number of new-formed a.v.as. with morphological features similar to those of the normal border. Their course, shape and length were modified in relation to the changed situation of the main arterial and venous trunks, and they appeared more tortuous and convoluted, and generally with a smaller calibre (Text-fig. 3; Pl. 1, fig. 6). Some of these a.v.as. present a coiled course which is less frequently recognizable among the normal shunts. A.v.as. in the form of termino-terminal inosculations are also present, but with a reduced diameter. An intense proliferation of capillary vessels was also noticed. Observations conducted after long periods of time (3, 4 and 8 weeks) did not show particular structural modification worthy of note. Practically, the definitive aspects of the regeneration appear within 2 weeks.

Serial sections of the resected border of the ear, cut in different planes, and examined after 2-3 weeks from operation, show the presence of small areas of granulation tissue. Vessels of 30-40 μ in diameter can be seen which often present a convoluted course and epithelioid differentiation of the media. Some of these are intermediate portions of a.v.as. with very narrow lumina, the diameter of these measuring about 10 μ . Small arteries of 80-90 μ diameter with a longitudinal layer of smooth muscle fibres disposed between the intima and the normal circular musculature of the media are also frequently found. From such arteries intermediate portions of a.v.as. often arise. It is noteworthy that the epithelioid modification is less frequent in both the a.v.as. and the arteries in the normal marginal portions of the ear. Arteries and veins with normal histological structure of the wall are also present.

DISCUSSION

Before considering and discussing the results of this investigation some critical observations about the technique employed in this work must be made. As has been stressed by others, in order to obtain satisfactory preparations, the neoprene-latex injections require the use of pressures which are notably higher than the physiological pressure. Hence it might be assumed that the vascular bed must undergo alterations in its original structure. It is reassuring, however, that in the cast preparations the capillary net is not damaged and that no extravascular injection mass appears in the interstitial tissue, as sometimes happens even with indian ink injections executed at pressures which are considerably lower than those used for the neoprene-latex method. Probably a part of the high pressure

employed in this technique is quickly exhausted against the wall of the larger arteries (in this case the carotid artery and its ramifications); the injection mass, possibly because of its limited fluidity and its high viscosity, reaches the capillary bed at a pressure which can be tolerated by the thin capillary walls. A control examination of the vascular system of organs nearer the injection point, such as



Text-fig. 3. New-formed arterio-venous anastomoses in the ear border following removal of the dichotomic ramifications to the edge. A.v.as. (solid black), arteries (white), veins (stippled). $\times 20$.

the tonsil, the nasal mucosa and the carotid body, made evident the possibility of a normal successful injection even in the capillary nets. In the rabbit's ear particularly, the a.v.as. themselves may play a part in lowering the pressure of the injected mass by offering to the liquid flowing in the arteries easier passages to the venous system. Certainly the neoprene-latex technique allows a sure and easy identification of the a.v.as. in the rabbit's ear. Further, the study of the connexions

and distribution of the vascular arrangement is simplified by this technique. Any doubt on the origin and on the connexion of small arterial branches with veins can easily be clarified in cast preparations examined under a binocular microscope and using a simple glass needle.

Undoubtedly the diameter of the cast corresponding to the lumina of the vessels is larger, as has been shown by the figures relating to the calibre of the capillaries, which is two to three times those of the normal capillary vessels. Certainly such an enlargement factor must be taken into account in arriving at an idea of the real diameter of the vessels and especially of the a.v.as.

In the study an account has been given for the first time of the details of the distribution of the arteries and veins in the rabbit's ear. This account is factual and does not require discussion. The a.v.as. in the rabbit's ear have also been re-examined: with regard to their structure, their course and their connexions, the description given here agrees with those of previous observers. From the cast preparations it is quite clear that the a.v.as. originate, in the main, from the smaller arteries, but they seldom arise from vessels with diameters of over 300μ . Frequently arterio-venous connexions were observed between a single arteriole and several different venules. The division of an arteriole into two branches, of which one is in direct communication with a vein and the other resolves into capillary vessels, as has been described by Vastarini-Cresi (1902) and Clara (1927), was rarely seen. Sometimes from the first portion of an a.v.a. there originates a small branch with capillary ramification, as was pointed out by Staubesand & Genschow (1952). However, a classification of the a.v.as. ignoring the structure and considering only the course, calibre and length appears rather difficult from examination of my specimens.

Some a.v.as., situated in the border of the ear, possess special features such as large lumina and a termino-terminal form of inosculation. In these cases the lumina are almost three times those of the normal a.v.as. scattered in the central and lateral areas of the ear. If one considers then that, as Clark (1938) indicated, 256 times the volume of blood flowing through a capillary of 10μ in diameter passes in the same time through an anastomosis 40μ in diameter and of the same length, it will be evident what a large and rapid passage to the blood proceeding from the arteries to the veins these particular anastomoses can offer. In this marginal portion of the ear, the speed and pressure of the venous blood circulation may differ little in range from the corresponding velocity and pressure in the arteries, since the blood flows in a circuit in which the variations in diameter of the arterial and venous vessels directly connected are very small. Furthermore, the fact that the arteries and arterioles of the rabbit's ear show irregular, periodic contractions at different rates, numbering from 2 to 12 per min., as has been long known, must be taken into account. The investigations of Clark & Clark (1934) demonstrated that the a.v.as. likewise show spontaneous and periodic contractions, with a rhythm independent of that of the arteries and of neighbouring a.v.as. and with a speed of contraction which is one-third that of the arteries. These irregular activities cause enormous variations of velocity, pressure and direction of the blood flow in the arterial as well as in the venous system. Both systems, however, with their numerous annular anastomoses, can easily respond to all these circulatory

variations and even permit a reversal of flow, thus preventing eventual local stasis. The '*sperrarterien*', here described, must play an important role in the distribution of the blood to different areas. Only the dichotomous arterial branches to the edge of the ear are, by their disposition, incapable of responding to the circulatory variations above mentioned; but in connexion with these vessels the anastomoses are larger, more numerous and constitute a countless series of shunts between the two systems.

With regard to the experimental regeneration of a.v.as., it must be remembered that the spontaneous formation of new a.v.as. has already been seen in living animals by Clark & Clark (1934). These investigators observed that the 'basis for their formation is universally present in the primitive arterial-venous capillary connexions and that the a.v.as. form within few days from small vessels already present in the vascular plexus by enlargement of the lumen, straightening of their course and thickening of their wall, through the addition of extraendothelial cells resembling the smooth cells on the arterioles'. Clark & Clark found that sudden temperature changes, infections and mechanical stimuli promote the formation of new a.v.as. In my experiments the resection of a small portion of the ear border, including the dichotomous ramifications of arteries and veins between which lie a great number of anastomotic shunts, determines a modification of the local blood circulation favourable to an intense regeneration of a.v.as. with the characteristics illustrated above. The diameter of the large arterial and venous branches remains almost unchanged, probably because of the pressure existing in the trunks from which they arise.

It appears legitimate to conclude that, as in the experiments of the Clarks, the regeneration of a.v.as. following removal of a portion of the ear is based mainly on pre-existing arterio-venous capillary connexions. These experimental observations appear to demonstrate that the development of a.v.as. is closely connected with the conditions existing in the local blood circulation.

SUMMARY

The vascular arrangement of the rabbit's ear has been re-examined by means of the neoprene-latex injection technique and the usual histological methods. The arterial and venous patterns of this organ were studied in thirty-five animals of different ages. Both these vascular systems possess an annular anastomotic disposition in connexion with which there exists a large number of arterio-venous anastomoses. An account of the distribution, number and shape of these arterio-venous anastomoses is given. Particular attention was paid to the a.v.as. situated in the ear border which present a large diameter and a termino-terminal inosculation form.

Observations on the experimental regeneration of a.v.as. of the ear are also recorded. Finally, some critical considerations concerning the vascular arrangement of the rabbit's ear and the regeneration of a.v.as. are presented.

I am greatly indebted to Prof. J. D. Boyd, not only for facilities provided, but also for criticism in the preparation of this paper.

REFERENCES

- BOYD, J. D. (1939). Arterio-venous anastomoses. *Lond. Hosp. Gaz. (Clinical Suppl.)*, **42**, 1-8.
- CLARA, M. (1927). Die arterio-venösen Anastomosen der Vögel und Säugetiere. *Ergebn. Anat. EntwGesch.* **27**, 246-301.
- CLARK, E. R. (1938). Arterio-venous anastomoses. *Physiol. Rev.* **18**, 229-247.
- CLARK, E. R. & CLARK, E. L. (1934). Observations on living arterio-venous anastomoses as seen in transparent chambers introduced into the rabbit's ear. *Amer. J. Anat.* **54**, 229-286.
- GRANT, R. T. (1930). Observations on direct communications between arteries and veins in the rabbit's ear. *Heart*, **15**, 281-303.
- HOYER, H. (1877). Ueber unmittelbare Einmündung kleinster Arterien in Gefäßäste venösen Charakters. *Arch. mikr. Anat.* **13**, 603-644.
- KRAUSE, W. (1884). *Anatomie des Kaninchens*. Leipzig: Verlag von Wilhelm Engelmann.
- PRICHARD, M. M. L. & DANIEL, P. M. (1953). Arterio-venous anastomoses in the tongue of the dog. *J. Anat., Lond.*, **87**, 65-74.
- ROSSATTI, B. (1954). Über die Blutzirkulation und die arteriovenösen Anastomosen der menschlichen Nasenschleimhaut. *Anat. Anz.* **100**, 243-247.
- SCHUMACHER, S. (1907). Über das Glomus coccygeum des Menschen und die Glomeruli caudales der Säugetiere. *Arch. mikr. Anat.* **71**, 58-115.
- SCHUMACHER, S. (1938). Über die Bedeutung der arteriovenösen Anastomosen und der epitheloiden Muskelzellen (Quellzellen). *Z. mikr.-anat. Forsch.* **43**, 107-130.
- STAUBESAND, J. & GENSCHOW, C. (1952). Die arterio-venösen Anastomosen im Löffel des Kaninchens nach graphischen Rekonstruktionen. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **116**, 446-457.
- STOLZENBURG, H. J. (1937). Experimentelle Untersuchungen über das Verhalten der arterio-venösen Anastomosen. *Z. mikr.-anat. Forsch.* **41**, 348-358.
- TISCHENDORF, F. (1938). Experimentelle Untersuchungen zur Histo-Biologie der arterio-venösen Anastomosen. *Z. mikr.-anat. Forsch.* **43**, 153-178.
- VASTARINI-CRESI, G. (1902). Comunicazioni dirette fra le arterie e le vene (anastomosi artero-venose) nei mammiferi. *Monit. zool. ital.* **13**, 136-142.

EXPLANATION OF PLATE

All figures are photographs of neoprene-cast preparations. The a.v.as. are indicated by arrows; the arteries by *a.*; the veins by *v.*

Fig. 1. General vascular arrangement of the rabbit's ear. $\times \frac{3}{4}$.

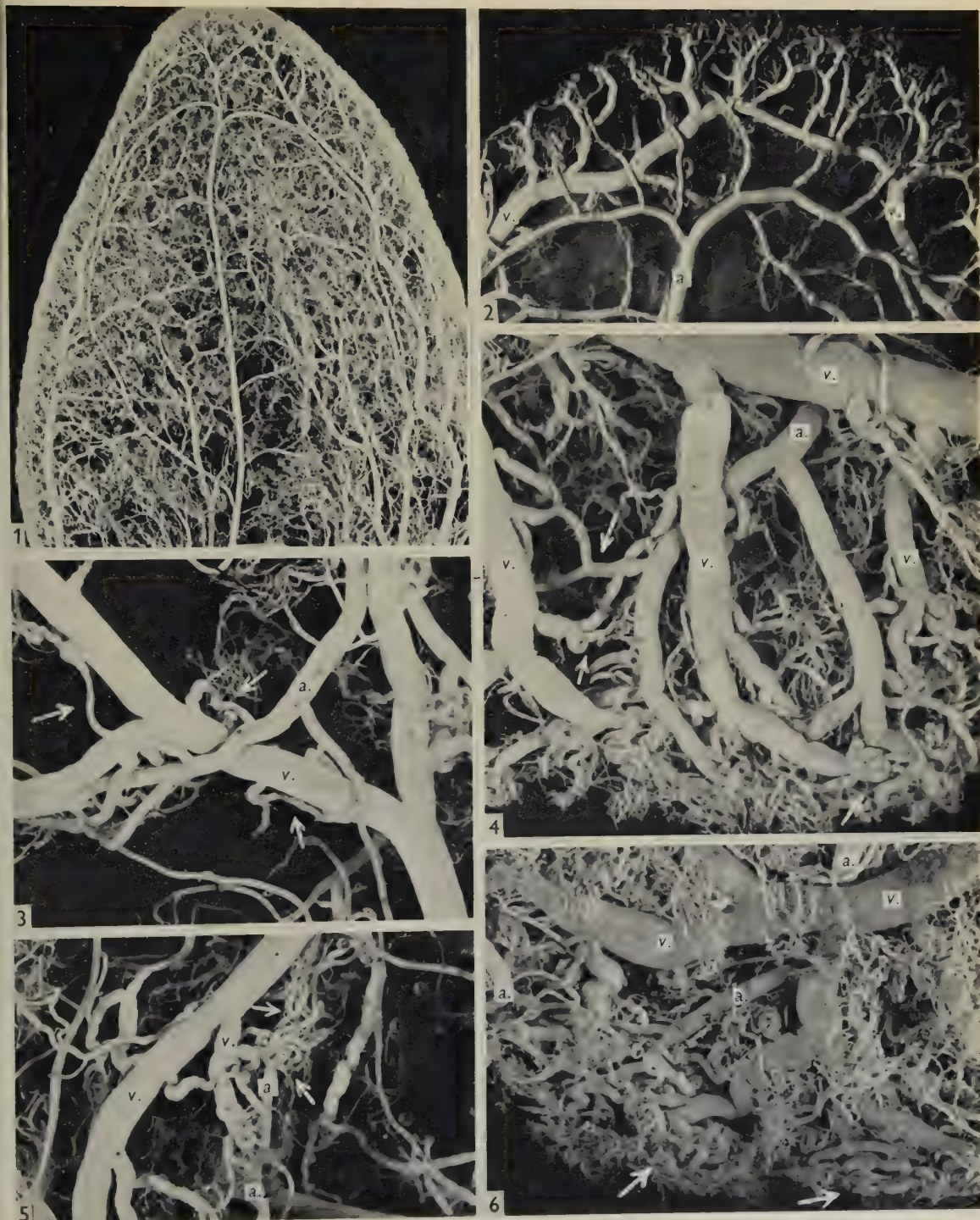
Fig. 2. Disposition of the arteries and veins directed to the edge of the ear. $\times 4$.

Fig. 3. A.v.as. of the central region of the ear. The shunts are near the point of contact of the two vessels. $\times 10$.

Fig. 4. A.v.as. from the dichotomous branches of the ear border. Some of these possess a termino-terminal inosculation form. $\times 10$.

Fig. 5. Branching a.v.as. with convoluted course in the central region of the ear. $\times 10$.

Fig. 6. New-formed a.v.as. in the ear border following resection of the dichotomous branches of the edge. They appear more convoluted and tortuous. $\times 10$.



BRUNO ROSSATTI—RABBIT'S EAR AND NEW-FORMATION OF ARTERIO-VENOUS ANASTOMOSES

(Facing p. 328)

OBSERVATIONS ON THE CAPILLARY BLOOD VESSELS OF THE HUMAN NAIL FOLD

BY E. W. WALLS AND T. J. BUCHANAN

Departments of Anatomy and Physics, Middlesex Hospital Medical School

'The extension of new capillaries by the formation of vascular buds not only provides the basis for the growth of blood vessels during normal development, it is also an essential process in the adult in the adjustment of the peripheral circulation to varying functional demands, and in the vascular reactions to inflammatory stimuli. There is evidence to show that, under normal stable conditions, new capillaries are continually being formed in this way while others may be retracted and absorbed, so that the capillary pattern in any part of the body is quite plastic and capable of alteration from time to time in response to the slightest changes in the immediate environment.'

This quotation from Le Gros Clark (1952) gives in brief the conclusions to be drawn from the work of Clark & Clark (1932) on the rabbit's ear. However applicable these conclusions may be in general, there seems little doubt that with regard to the capillary blood vessels of the human nail fold the state of affairs described above does not obtain. Not only have a number of workers in this field stated that there is a considerable degree of constancy in the pattern of these vessels, but it has even been claimed that over periods, certainly of months, they may be as characteristic of the individual as finger prints (Braasch & Nickson, 1948). It would appear, moreover, from the work of Staple (1955) on the gum that the nail fold is not unique in this respect.

Of recent years the increasing use of radioactive substances has focused attention afresh upon these small vessels of the fingers, for it has been established that whatever other agencies may effect a change in their pattern, irradiation by X-rays assuredly can (Arons, Freeman, Sokoloff & Eddy, 1954). With this in mind it is clearly of importance to be sure of two things: first, that the high degree of constancy of appearance claimed for the nail-fold capillaries is in fact true and, secondly, the extent to which their pattern may be altered by external factors other than X-irradiation.

The present paper is mainly concerned with answering the first question, but certain observations made with regard to the second seem of sufficient interest to be included.

If at this point it is allowed that in health the nail-fold capillary pattern is constant over periods of months, two conclusions follow: not only must the vessels remain unchanged in number, length and calibre, but also they must all be patent and carrying blood (plasma and corpuscles) each time they are examined. Now, capillaries elsewhere in the body are not constantly open but, on the contrary, as circumstances demand, vary between full dilatation and complete constriction. It is the opinion of Krogh (1922) and of Crawford & Rosenberger (1926) that all the

nail-fold capillaries are usually open, a view which has recently been strengthened by the findings of Braasch & Nickson (1948) who failed to observe these vessels open and close as do capillaries in other sites. This point will be returned to later.

The question of pattern alteration in disease will not be discussed in this paper, but it should be pointed out that claims have been made that certain neurological and neuropsychiatric disorders are accompanied by such alteration (Hauptmann, 1946; Castellanos & Gibson, 1950).

MATERIALS AND METHOD

Observations were made on sixty-eight apparently normal people, sixty-three aged 18–30 years and five aged 40–50 years.

The apparatus used was, in principle, similar to that described by Crooks (1953). After washing with soap and water, followed by drying with alcohol, the finger to be examined was placed vertically in a Perspex trough containing liquid paraffin, with its tip resting lightly on the bottom of the trough. This arrangement not only gave adequate stability to the finger, but also conferred the great advantage of complete freedom from high-lights. A microscope, with eyepiece and draw-tube removed, was mounted with its axis horizontal in front of the finger, and a reflex camera, with lens removed, was connected to the microscope body tube by a metal cylinder 34 cm. in length. Diaphragms were inserted in this cylinder at suitable intervals to eliminate internal reflexions. Focusing was obtained by the usual rack and pinion microscope adjustment. The lens used was a Leitz Summar fitted with an adjustable iris diaphragm, the overall magnification obtained being times 18. Illumination was provided by four Baker microscope lamps fitted with 6 V., 30 W. bulbs, arranged round the trough to give uniform lighting of the nail fold. Heat-reducing filters were found to be essential.

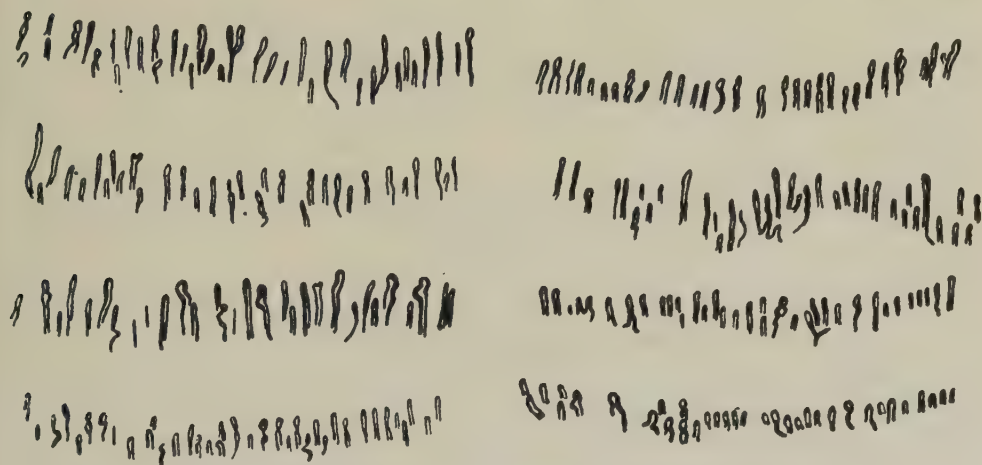
After a good deal of experiment the most satisfactory photographs were obtained by using Ilford Selochrome plates with an exposure of $\frac{1}{12}$ sec., followed by development for 2 min. at 18° C. in Johnson's contrast developer.

FINDINGS

(1) *General.* It must be stated at the outset that many of the subjects examined did not show clearly discernible capillary patterns in their nail folds. While in most subjects it was possible, by direct microscopic examination, to see some capillary vessels, as far as making photographic records was concerned only one subject in five gave results of a really high quality. This was of great importance in selecting subjects suitable for a long-term study, for in comparing photographs of the same pattern taken at intervals of weeks or months the need for fine detail was quite essential. The point was of less importance when only a general idea of pattern was required, as this could be obtained from photographs of less clearly marked fields. In nearly all subjects the ring and little fingers, particularly the ring, gave clearer views of the nail-fold vessels than either the middle or index fingers. In certain subjects the capillaries could be seen more easily on some days than others. The reason for this was not discovered, but it is probable that differences in the degree of sweating at the time of examination were responsible.

As seen under a low-power microscope, or on the ground-glass screen of the camera, the nail fold shows as a curved zone formed of epidermis distally and dermis proximally. The epidermis, translucent and insensitive, is moulded on the dermal papillae which may be very variably developed in different subjects. Vascular loops, which together make up the capillaries of the terminal row, run into the papillae but stop some way short of the epidermal-dermal junction (Pl. 1, fig. 6). Each loop consists of an afferent arterial limb and an efferent venous limb, the flow of blood always being in the same direction—from the narrow arterial side of the loop to the wider venous side. The speed of flow, which varies considerably in different loops, is usually most rapid in those of smallest calibre. Figures quoted by Braasch & Nickson (1948) for the diameter of the limbs of the loops are arterial limb $9-12\mu$ and venous limb $9-20\mu$. These are in general agreement with those found in the present investigation.

(2) *Range of pattern.* Outline drawings of the terminal row capillaries of eight healthy subjects aged 20 to 30, selected at random, are shown in Text-fig. 1, and

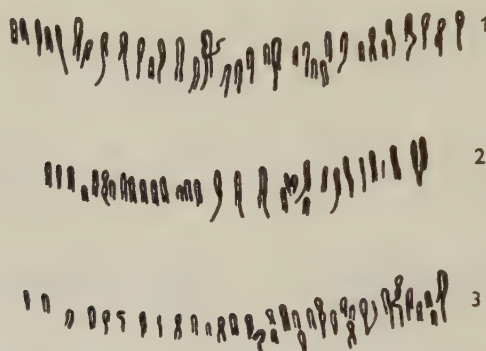


Text-fig. 1. Tracings of photographs of the terminal row capillaries of the left ring fingers of nine subjects aged 20 to 30, to show the range of pattern.

it will be seen that the range of pattern is considerable. Both in shape and size the capillaries showed wide variations between different subjects, an observation which is in agreement with the findings of Millar & Harriman (1950) and of several earlier workers. It is quite true that in some cases the terminal row capillaries were long and hair-pin like, and were arranged in a regular manner parallel to one another (Pl. 1, fig. 1); in others, however, the vessels appeared quite short, either because their length was actually less or because they were approaching the surface more vertically (Pl. 1, fig. 5). Hauptmann (1946) considers that when more than 20 % of the capillaries in a given field consist of forms other than the hair-pin type, i.e. show tortuosity or slight twisting, or form figures of eight, the pattern should be considered abnormal. This view will not be disputed here, but certain observations may be relevant. First, the occurrence in varying degree of tortuosity or figure-of-eight formation in the terminal loop of some capillaries was a very common

finding in the present study (Pl. 1, fig. 3); secondly, the degree of tortuosity or other form of deformation may vary with time (Pl. 1, figs. 2, 3); and finally, the question of whether a particular vessel should be designated tortuous or slightly twisted is by no means always clear cut. In brief, it would appear to the authors that the qualitative criteria, upon which quantitative assessments of normality of capillary pattern are based, are far from absolute and as such open to challenge.

It might be thought that the different fingers of the same individual would in health show comparable patterns whereby general systemic effects on these small vessels might be assessed. This is the opinion of Hauptmann (1946), who believes that a person's capillary pattern is usually the same in all fingers of both hands. In the present investigation this was not always found to be the case, for not only might different fingers of the one hand show patterns of obvious dissimilarity, but the comparable fingers of the two hands of the one individual when examined within a few minutes of each other were in some cases strikingly different in capillary pattern (Text-fig. 2); and indeed even in the same nail-bed the capillaries



Text-fig. 2. Tracings of photographs, made within a few minutes of each other, of the left ring finger (1), left little finger (2) and right ring finger (3) of a male aged 30.

were not always of uniform appearance. The importance of these observations in assessing the normality or abnormality of pattern in clinical studies is obvious, but clearly they cannot be held to make such an assessment other than very difficult. Indeed they might go some way to explaining the conflicting reports which have appeared with regard to the alteration of pattern in, for example, disseminated sclerosis (Millar & Harriman, 1950; Mutlu, 1951).

Irrespective of the pattern presented by the nail-fold vessels, it is of interest to note that the number of capillaries in the terminal row was remarkably constant at approximately nine per mm. In the usual field photographed this gave a total of some thirty capillaries; deviations from this figure of more than 10% were seldom found.

The age range of the subjects examined in the present study was too restricted to permit of any conclusions being drawn with regard to the effect of age, nor, although twenty of our subjects were females, was any difference in pattern detected which might be correlated with sex.

(3) *Constancy of shape and pattern.* When examined over a period of months the most striking feature of the nail-fold capillaries is the considerable degree of constancy which they show in shape and position (Pl. 1, figs. 2-6, 8-10). The pattern of a given finger generally has its own distinctive features which make identification a simple matter. In order to determine the degree of permanence of the pattern, photographs of the left ring finger were taken at intervals in five subjects over periods ranging from 6 months to 1 year. In all cases the patterns were readily recognizable at the end of the period of examination, but close scrutiny of the series of photographs showed that three types of variation in pattern could be detected.

(a) In three cases a curious variation in the length of the capillaries of the terminal row was noted (Pl. 1, figs. 4-6, 8-10). Maximum and minimum measurements differed by as much as 50 %, far too great to be accounted for by accidental tilt of the finger in the trough, whereas the lateral separation of the capillaries remained unaltered. To check the possible effect of finger tilt within the trough a series of photographs was taken with the finger held in three positions: first vertical, then tilted as far forwards as possible and finally tilted as far backwards as possible. The difference in capillary length detectable in the three photographs was so slight as to rule out all possibility of finger tilt as a causative factor. Manicure of the nail fold was not practised by any of the subjects in whom this change was detected. In one subject, in whom both ring fingers were photographed at intervals, the change of length in the vessels was noted to occur in both fingers, but not at the same time; this would seem to indicate a purely local origin of the change which may result from a mechanical effect on the tissues of the nail fold caused by growth of the nail. Whatever its cause, however, the importance of the finding is considerable, indicating as it does that the permanence of the capillary pattern is in fact greater than a random pair of photographs might show. If, for example, only two photographs were taken, at, say, 1 month's interval, the second might show an apparent change which in reality is only transient, the pattern extending and contracting about a certain mean length.

(b) An examination of the photographs, in some cases, revealed complete disappearance of certain capillaries (Pl. 1, figs. 2, 3). In these subjects there is little doubt that the changed appearance was due, not to slight alterations in the plane of focus, but either to a complete absence of blood from the vessels in question or to the presence within them of plasma devoid of red blood corpuscles. This explanation is supported by the fact that capillaries, which in some photographs appeared quite full of blood, in others could only be identified by a series of dots corresponding to their reduced content of red cells at that instant. To what extent such variations in capillary visibility depend upon capillary contractility is a vexed question and need not be discussed here. What can be stated, however, is that some individuals show this feature very clearly while others, the majority, seldom show it at all. These findings, based on photographic records, have been supported by direct microscopic observation of the blood flow in the nail-fold vessels. The movement of the red blood corpuscles—with here and there amongst them a leucocyte—is easily seen, and the general picture presented is that of dilated active capillaries. Occasionally, however, a small capillary may disappear for a few seconds, or the

flow within it may decrease to the point at which only a few corpuscles can be seen moving along.

(c) In some subjects a change could be detected in the shape of individual capillaries (Pl. 1, figs. 8, 10). Such changes persist, and there can be no doubt that they are of a different nature from those described above, taking place slowly over periods of months. The changes noted under (a) and (b) are temporary and must be recognized as such. Those noted under (c) are genuine alterations of pattern, but they are small changes affecting individual capillaries, and close study may be required to detect them.

In summary of what has been said it may be stated that over a period of a year the basic pattern of the nail-fold capillaries presents a high degree of constancy. Accordingly, it was decided to attempt to alter the pattern by interfering with the finger in a not too drastic manner.

THE EFFECT OF BLISTERING

Two methods of producing a blister over the area of the terminal capillary row were employed. In three subjects a stick of carbon dioxide snow was applied to the nail fold for 45 sec. Within a few minutes the area became red and swollen, considerable pain being experienced during the process of thawing out, and by the next day a well-marked blister had formed in two of the subjects. After several weeks, when the region had apparently returned to normal, photographs were taken of the nail-fold vessels and compared with those taken immediately prior to freezing. In all three cases a close comparison showed that vessel for vessel the terminal row capillaries were very nearly all still recognizable (Pl. 1, figs. 6, 7, 11 and 12). Although somewhat shorter, and in one case slightly tortuous compared with their previous quite straight appearance, there was no difficulty whatsoever in identifying the fields.

In three further subjects large blisters were raised by the application of a cantharides plaster to the area of the nail fold for a period of 12 hr. (It is of interest to note that in two additional subjects such treatment failed to cause blistering; that fact, together with the fear of producing systemic effects by the use of cantharides, led to the method being abandoned in favour of carbon dioxide as described above.) In order to visualize the capillary bed immediately, the blister was carefully removed in these cases as soon as it had fully formed. This of course gave a very clear view of the vessels, but on reflexion it was felt that the slight mechanical trauma thereby caused may have been responsible in these cases for a less perfect return to the original pattern than in those treated with carbon dioxide, and thereafter left undisturbed. Even so, the tendency for the nail-fold capillaries to retain their pattern was very noticeable in two of the three subjects (Pl. 1, figs. 11, 12). In the third case the restored pattern, though in general form similar to the original, showed several differences in detail which were thought to be due to an incidental injury, accompanied by bleeding, suffered during the period of healing.

CUTTING

Following a cut through the base of the nail fold, made so as to sever the terminal row of capillaries, it could scarcely be expected that the subsequent pattern would faithfully reproduce the original. However, in four subjects in whom this small operation was carried out the final pattern was in general form closely similar to the original. Thus if the pattern before cutting had been composed of long vessels then long vessels made up the final pattern and vice versa. To that extent at least then the pattern showed a strong tendency to constancy. In one subject an interesting result was the presence in the final pattern of capillaries growing backwards towards the root of the finger. Amongst the great majority of vessels passing distally these few vessels which took a proximal course made a striking picture (Pl. 1, fig. 13).

The conception of the capillary pattern as something plastic which is capable of alteration in harmony with local conditions does not appear to receive much support from the observations made on the human nail fold. Structurally the capillaries of this region appear to maintain a surprising constancy over long periods, as do the similar vessels of the human gum (Staple, 1955). If the capillaries of these two areas are unique in this respect—and they may not be—an explanation might be sought in the character of their supporting tissues compared with those of similar vessels elsewhere.

SUMMARY

1. The capillary vessels of the nail fold have been examined in sixty-eight normal subjects.
2. The range of capillary pattern met with in different subjects is considerable.
3. The pattern in a given finger is remarkably constant over periods of up to 1 year.
4. Variations in the appearance of the pattern, which are of a temporary nature, occur and are described.
5. Changes of a permanent character affecting individual vessels also occur, but these are of very gradual development.
6. The effect of blistering on the nail-fold vessels is described.

We extend our sincere thanks to the medical students and others who helped in this investigation, and to Mr P. Runnicles who, besides helping in many other ways, also prepared the photographs.

REFERENCES

- ARONS, I., FREEMAN, J., SOKOLOFF, B. & EDDY, W. H. (1954). Bio-flavonoids in radiation injury. I. The effect of ionizing radiation on capillaries. *Brit. J. Radiol.* **27**, 583–585.
- BRAASCH, N. K. & NICKSON, M. J. (1948). A study of the hands of radiologists. *Radiology*, **51**, 719–727.
- CASTELLANOS, M. & GIBSON, W. C. (1950). Abnormality of the capillary nail bed. *Arch. Neurol. Psychiat., Chicago*, **63**, 140–142.
- CLARK, E. R. & CLARK, E. L. (1932). Observations on living preformed blood vessels in the rabbit's ear. *Amer. J. Anat.* **49**, 441–477.
- CLARK, W. E. LE GROS (1952). *The Tissues of the Body*, 3rd ed. p. 198. Oxford: Clarendon Press.
- CRAWFORD, J. H. & ROSENBERGER, H. (1926). Studies on human capillaries. II. Observations on the capillary circulation in normal human subjects. *J. clin. Invest.* **2**, 351–364.

- CROOKS, H. E. (1953). Photography of epidermal ridges and superficial blood capillaries of the fingers. *Med. Biol. Illust.* **3**, 198–205.
- HAUPTMANN, A. (1946). Capillaries in the finger nail fold in patients with neurosis, epilepsy and migraine. *Arch. Neurol. Psychiat., Chicago*, **56**, 631–642.
- KROGH, A. (1922). *The Anatomy and Physiology of Capillaries*. New Haven: Yale University Press.
- MILLAR, J. H. D. & HARRIMAN, D. G. F. (1950). The nail bed capillaries in disseminated sclerosis. *J. Neurol. Psychiat.* **13**, 312–313.
- MUTLU, N. (1951). Capillaroscopic studies in cases of multiple sclerosis and other neuropsychiatric disorders. *Arch. Neurol. Psychiat., Chicago*, **66**, 363–367.
- STAPLE, P. H. (1955). Observations on the gingival capillary circulation in human subjects (abstract). *J. dent. Res.* **34**, 783.

EXPLANATION OF PLATE

All figures are photographs of the nail fold of the left ring finger. The magnification of fig. 1 is twice that of figs. 2–13.

Fig. 1. Female, aged 40. General view of the nail-fold vessels.

Figs. 2, 3. Female, aged 20. Photographs taken 9 September 1954 and 27 May 1955. The constancy of pattern is apparent, although minor variations in shape may be detected in individual vessels. Moreover, in fig. 3 the 9th vessel from the left can be seen to have a double loop not visible in the earlier photograph. An explanation for this finding is offered in the text.

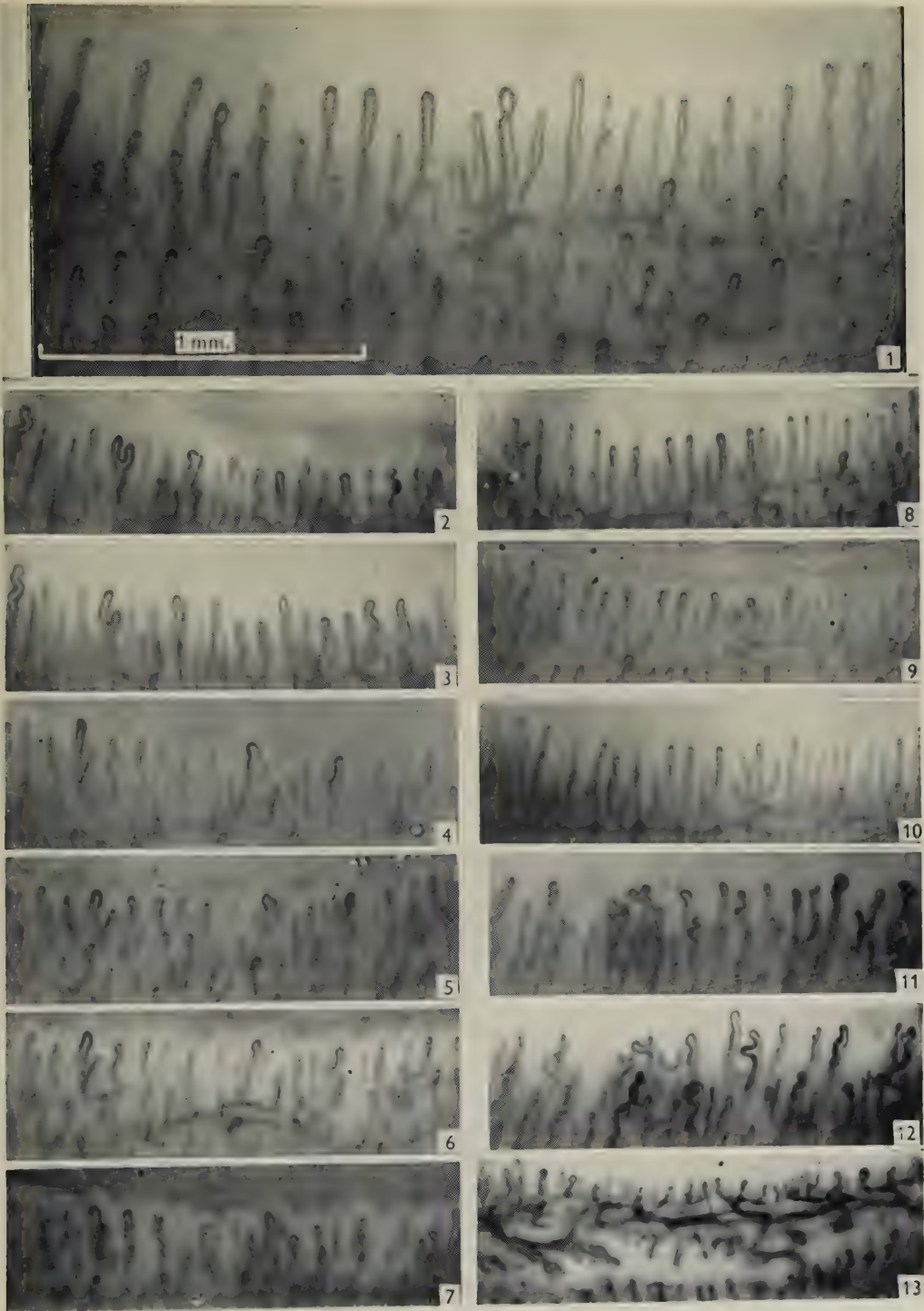
Figs. 4–6. Male, aged 30. Photographs taken on 29 October 1954, 10 May 1955 and 15 July 1955. It can be seen that while figs. 4 and 6 show patterns which are virtually identical, fig. 5 seems at first sight to be noticeably different. In fact, the pattern in fig. 5, on close inspection, is seen to be the same as in figs. 4 and 6, but the length of the individual vessels is considerably less. Clearly when fig. 5 was taken the nail-fold vessels were in a short phase.

Fig. 7. The same field as in figs. 4–6, photographed on 15 September 1955, i.e. 9 weeks after blistering with carbon dioxide. The retention of the original pattern can be recognized when comparison is made with fig. 5.

Figs. 8–10. Female, aged 40. Photographs taken on 9 September 1954, 21 December 1954 and 2 June 1955. The general constancy of pattern, apart from minor variations in individual vessels, is apparent. Compared with figs. 8 and 10 the vessels in fig. 9 can be seen to be in a short phase.

Figs. 11, 12. Male, aged 21. Photographs taken on 22 December 1954 (before blistering) and on 1 April 1955 (fig. 12), 14 weeks after blistering with cantharides. The retention of pattern, despite the severe blister which was removed, is obvious.

Fig. 13. Male, aged 49. Photograph taken 12 weeks after cutting the nail fold. Some regrowth of capillaries in a proximal direction is shown.



WALLS AND BUCHANAN—CAPILLARY BLOOD VESSELS OF THE HUMAN NAIL FOLD

(Facing p. 336)

ON THE INDIVIDUAL VARIABILITY OF FIBRE COMPOSITION IN HUMAN PERIPHERAL NERVES

BY J. TOMASCH AND W. A. BRITTON

Department of Anatomy, Queen's University, Kingston, Ontario

In a previous study one of us investigated the fibre composition of a number of muscle nerves and cutaneous nerves in one individual, and we found that these two types of nerves are distinctly of different composition (Tomasch & Schwarzacher, 1952). However, in this individual, all the muscle nerves were quite similar, and so were all the cutaneous nerves. We found recently that the same arrangement exists in the nerve supply to the larynx, where the fibre composition of motor and sensory branches were investigated (Tomasch & Britton, 1955). In the work presented here we tried to determine the range of variation of this difference between the two types of nerves.

Twelve autopsies on individuals free of any neurological disorder provided the source of the material investigated. The material was selected with the view to having different ages represented, preferably two subjects of each decade. From each of the twelve post-mortem cases available a cross-section of the medial popliteal nerve in the popliteal fossa was taken. Similarly, sections of the sural nerves and muscular branches to the gastrocnemius muscle were taken at the same level.

After embedding in paraffin, the sections were cut at a thickness of 5–10 μ . The sections were stained using the Häggqvist modification of the Alzheimer-Mann glia staining method. From the sections selected, the bundles chosen for investigation were photographed using a photomicrographic apparatus in connexion with a miniature camera. Sufficient photographs were taken to enable us to count and measure about 500 fibres in each bundle. As shown by Häggqvist (1936) such a number of fibres is necessary to obtain a reliable representation of the fibre composition. The photographs were analysed by measuring in microns the diameter of the fibres they contained, while simultaneously re-locating their presence microscopically on the original section. The initial magnification was $\times 400$; the final magnification on the photographic enlargements amounted to $\times 1500$. The actual measuring and counting was performed by first identifying each fibre on the original section, and then measuring its diameter on the photograph. The fibres counted were marked off on the picture. Not all fibres were round; some showed an oval shape. In these cases the smallest diameter was measured. Sometimes the fibres were pear-shaped, in which case the base of the pear was taken as the diameter. For polyhedral-shaped fibres an approximate round shape was reconstructed visually and measured. Where a fibre's diameter coincided directly with an even micron, it was recorded in the next higher interval. For example, a fibre showing a diameter of an even 2 μ could be placed in the 1–2 class or the 2–3 class. We preferred to place it in the higher class. No attempt was made by us to correct our values for the shrinkage involved in the

fixation and staining process. Arnell (1936) evaluated this shrinkage for the Häggqvist method at 28–29 %. The statistical problems involved in this technique have been dealt with by Häggqvist, Söggqvist (1938) and Hjiang (1950). The results obtained from single nerves or bundles were depicted in curves as customary in fibre analysis. On the abscissa the thicknesses of the fibres in microns were graduated; on the ordinate their percentage of (about) 500 fibres counted was given. The resulting curves are bimodal in human nerves, one spike usually in the $1\text{--}2\mu$ class, the second elevation usually in the $7\text{--}8\mu$ column. Although the height of these two elevations is fairly significant for the distinction of motor from cutaneous branches, we re-confirmed that most significance is contained in the so-called index of small fibres. This index is a value integrating the whole of the elevations rather than only comparing their height. It is obvious that the two elevations represent two so-called normal distribution curves which slightly overlap at the $4\text{--}5\mu$ column. The depression present there in the curves is referred to as the minimum. The index value is then obtained by adding half of the fibres of this minimum column to all the larger fibres

Table 1. *Muscular branches*

Age (years)	Maxima		Minimum (μ)	Largest fibres (μ)	Index
	Large fibres (μ)	Small fibres (μ)			
9	7–8	1–2	4–5	11–12	1.2
21	7–8	2–3	4–5	9–10	4.0
32	7–8	1–2	4–5	12–13	2.1
42	7–8	1–2	5–6	10–11	2.8
48	9–10	1–2	6–7	12–13	3.9
54	8–10	1–2	4–5	15–16	3.0
56	7–9	1–2	5	11–12	3.8
65	6–8	1–2	4–5	8–9	4.7
72	7–8	1–2	5–6	11–12	1.7
78	6–7	1–2	3–4	11–12	2.3

and letting this sum represent unity. The remaining half of this minimum column, plus all the small fibres, are then set in proportion to the number of large fibres. If the proportion is 4:1, this means that this nerve contains four times as many fibres of small calibre as of large calibre or, abbreviated, the index of this nerve is four. Accordingly, an index of five would indicate that there are five times as many small fibres as large ones. In every investigated case this index gives a clear and significant indication of the nerve's composition in this respect.

A further explanation is necessary concerning the representation of a nerve branch in one curve. Actually, most muscular or cutaneous branches are composed of a number of bundles of fairly uniform character. In order not to increase the number of curves unduly, we used for a single muscle branch or cutaneous branch only one curve that was averaged from those bundles which were large enough conveniently to be photographed.

The results of the measurements on the nerves investigated are shown in Tables 1–4. Two principal findings emerge from a study of the tables. In the first place previous findings regarding the difference in fibre content between muscular and cutaneous nerves in man are confirmed. Earlier papers, most of them resulting from work done on the nerves of animals other than man, have dealt with this subject in

a qualitative rather than, as we attempted, in a quantitative manner. The literature on this aspect has been dealt with in the paper by Tomasch & Schwarzacher and needs no repetition here. The second principal finding in our present work, which to our knowledge has not been shown previously, is the fact that human peripheral nerves, at least in the limbs, show a great variation in different individuals.

Table 2. *Cutaneous branches*

Age (years)	Maxima		Minimum (μ)	Largest fibres (μ)	Index
	Large fibres (μ)	Small fibres (μ)			
21	7-9	1-2	6	11-12	5.8
32	6-7	1-2	4	8-9	5.0
34	9-10	1-2	6-7	19-20	3.6
42	7-8	1-2	4-5	10-11	6.6
48	7-8	1-2	6	9-10	8.3
54	7-8	1-2	6	10-11	8.0
56	5-6	1-2	4-5	12-13	5.5
65	7-8	1-2	6	9-10	9.3
72	7-8	1-2	5	14-15	6.5
78	7-8	1-2	5-6	9-10	11.5

Table 3. *Bundles of medial popliteal nerves in 56-year-old male*

Bundle no.	Maxima		Minimum (μ)	Largest fibres (μ)	Index
	Large fibres (μ)	Small fibres (μ)			
1	7-8	1-2	5	12-13	4.0
2	8-9	1-2	5-6	12-13	4.6
3	7-8	1-2	6	10-11	4.7
4	7-8	1-2	5-6	9-10	4.8
5	8-9	1-2	5-6	11-12	4.8
6	8-9	1-4	6-7	11-12	5.0
7	8-9	1-2	5-6	9-10	5.4
8	7-8	1-2	6-7	11-12	5.4
9	9-10	1-2	6-7	10-11	5.7
10	8-9	1-2	6	10-11	5.7
11	8-9	1-2	6-7	12-13	6.0
12	7-9	1-2	5-6	11-12	7.2
13	8-10	1-2	6	12-13	7.6
14	7-9	1-2	6	9-10	8.3
15	8-9	1-2	5-6	10-11	8.4
16	7-8	1-2	5-6	9-10	9.2
17	8-9	1-2	6	12-13	9.5
18	8-9	1-2	6-7	11-12	9.6
19	9-10	1-2	7-8	12-13	9.8
20	7-8	1-2	6	9-10	11.6
21	7-8	1-2	6	10-11	10.5
22	9-10	1-2	6	10-11	10.8
23	7-9	1-2	6	9-10	14.1
24	9-10	1-2	6	11-12	14.6
25	8-9	1-2	6	9-10	15.0
26	8-9	1-2	6	10-11	17.6
27	None	1-2	None	11-12	None
28	None	2-3	None	10-11	None
29	None	1-2	None	11-12	None
30	None	1-2	None	9-10	None
31	None	1-2	None	10-11	None
32	None	1-2	None	9-10	None
33	None	1-2	None	8-9	None

The number of large fibres in bundles 27-33 was too slight to cause an elevation in the large fibre group.

The differences in fibre content between the two types of nerve are best demonstrated in a graph (Fig. 1), in which from each of our cases the indices of the muscular branch and the cutaneous branch respectively are plotted on the ordinate. The indices of the muscular branches from the various cases investigated, as indicated on the abscissa, are joined to form a curve and, similarly, the indices of the cutaneous branches. By the distance between them the two curves show very clearly that in

Table 4

Age (years)	Maxima		Minimum (μ)	Largest fibres (μ)	Index
	Large fibres (μ)	Small fibres (μ)			
	Medial popliteal nerves—bundles with most large fibres				
9	8-9	1-2	5-6	12-13	1.9
21	9-10	1-2	6-7	12-13	2.3
27	8-9	2-3	5-6	12-13	1.2
32	7-8	1-2	4	12-13	1.2
34	9-10	1-2	5	13-14	1.6
42	8-9	1-2	5	11-12	1.6
48	7-8	1-2	5-6	10-11	2.8
54	8-9	1-2	5	12-13	1.9
56	See Table 3				
65	9-10	1-2	5-6	12-13	1.8
72	6-7	1-2	4-5	10-11	2.1
78	7-8	1-2	5-6	12-13	3.8
Medial popliteal nerves—bundles of intermediate composition					
9	6-7	1-2	5-6	10-11	3.3
21	7-8	1-2	5-6	11-12	3.0
27	8-9	1-2	6	11-12	2.6
32	8-9	1-2	5-6	11-12	1.9
34	8-9	1-2	6	13-14	4.0
42	8-9	1-3	5	11-12	2.6
48	9-10	1-2	7	11-12	2.2
54	9-11	1-2	6	15-16	4.5
56	See Table 3				
65	10-11	1-2	7	12-13	3.8
72	9-10	1-2	7-8	11-12	6.7
78	6-8	1-2	5-6	9-10	9.8
Medial popliteal nerves—bundles with most small fibres					
9	6-7	1-2	5	9-10	8.7
21	7-8	1-2	5	9-10	10.0
27	7-8	1-2	6	10-11	9.8
32	7-8	1-2	6	11-12	6.2
34	9-10	1-2	5-6	15-16	6.3
42	7-8	1-3	5-6	10-11	8.6
48	7-8	1-2	6	10-12	8.8
54	9-10	1-2	6	14-15	13.9
56	See Table 3				
65	9-10	1-3	7-8	11-12	8
72	8-9	1-2	6-7	10-11	15.0
78	8-9	1-2	6-7	10-11	11.3

every case the number of small fibres is remarkably higher in the cutaneous branch than in the muscular branch. This is evident when we recall that the index is formed to indicate how many times as many small fibres as compared with those of large calibre are contained in a given nerve cross-section. Although in one individual some variation may occur between different muscular branches, such variation must be relatively slight. According to the investigation by Rexed & Therman (1948),

there is no difference between extensor and flexor muscle nerves in the cat. We therefore believe that in one given individual the muscular branches, at least of the limb muscles, are all of very similar composition. The basis for this belief is also derived from the investigation (Tomasch & Schwarzacher, 1952), in which several muscular and cutaneous nerves of one and the same individual of 48 years of age were analysed. This analysis showed the least number of small fibres of all subjects yet investigated, fewer than any in our present study. In the muscular branches in this individual there are fewer small than large fibres, and the index comes down to 0.8. For the cutaneous nerves the index rises to 2.9. Tomasch & Schwarzacher were inclined to believe, at that time, that these findings would apply to most individuals. This view was supported by the great similarity in the fibre composition of cranial

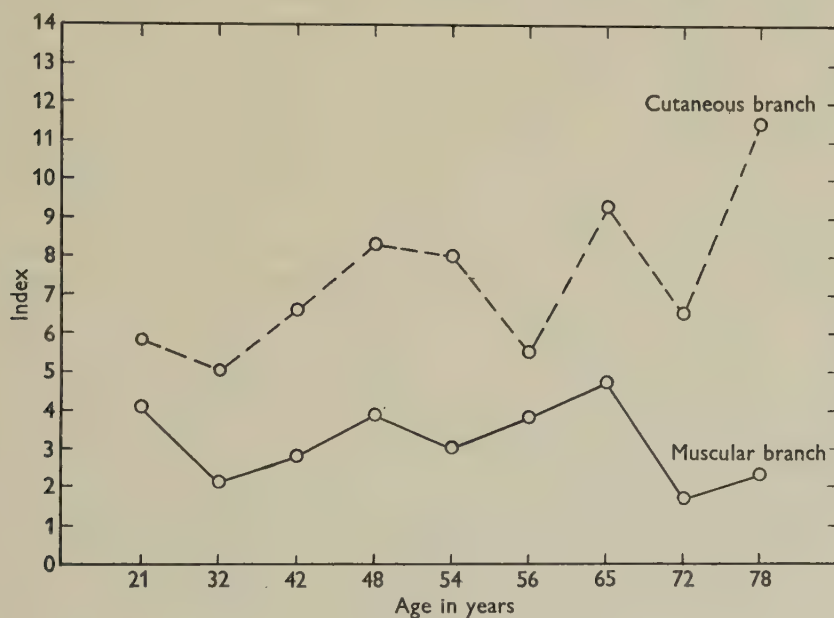


Fig. 1.

nerves and spinal roots found by different investigators. Using the identical technique in the work presented here we observed that in some individuals the number of small fibres is considerably higher; for example, in the individual of 65 years the index of the muscular branch, the highest we encountered, is 4.7, and as such considerably higher than any of even the cutaneous branches from the subject referred to above. Including the case analysed by Tomasch & Schwarzacher, the range of the indices for the muscle nerves we investigated is therefore 0.8–4.7, this range being obtained from ten subjects.

The functional significance of the existence of two fibre groups in the muscle nerves has been recently considered by Fernand & Young (1951). In their introduction they state '...the nerves to certain striated muscles contain large and small fibres, but few of intermediate size. The study of these fibre groups has been carried forward by various workers and especially by Sherrington himself, who in 1930

showed, with Eccles, that the large and the small fibre groups both contain afferent as well as efferent fibres. The significance of the differences in diameter of the fibre groups is still not well understood. The speed of conduction of nervous impulses is dependent on some factors correlated with the diameter of the fibres (Gasser & Erlanger, 1927), but it is not yet clear whether or how the conduction velocity of the distinct fibre groups affects their functioning. Presumably the conduction velocity is an important variable in determining the nature of the information carried by a nerve fibre.'

We find these statements, according to our own understanding of related literature, an excellent definition of to-day's status of knowledge on this matter. In their own investigation Fernand & Young used the nerves of rabbits, and found in the muscle nerves to the limb muscles maxima of both fibre groups. They speak of a bimodal type of curve, but found in nerves to the face, larynx, infrahyoid muscles and the diaphragm small fibres only, producing a unimodal type of fibre distribution curve. We ourselves analysed the fibres concerned in the nerve supply to the human larynx (Tomasch & Britton, 1955) and found the branches supplying its muscles to be distinctly bimodal in this respect, differing from those in the rabbit which were found by Fernand & Young to be unimodal. They found also the phrenic nerve in the rabbit to be of the unimodal type, while Tomasch & Schwarzacher in the human found it to be clearly bimodal. It is therefore doubtful if their classification of muscle nerves into unimodal and bimodal types will apply for the human subject. In physiological experiments conclusions are often based on findings obtained from study of animal nerves alone; in view of the above differences, however, certain reservations may have to be made regarding the use of data from animal nerves when considering problems of human nerve physiology.

This is particularly important in consideration of our second principal finding: that in different individuals, the composition of nerve branches varies considerably. It is therefore most likely that nerve impulses which in one individual, where the number of large fibres is high, are conducted by these large fibres must in another individual be conducted by small fibres when the large fibres are very scarce. It is not likely, in these cases, that a few large fibres will do the same amount of work as a great number in another individual, although this possibility has to be considered. The probability that functions carried out in one individual by large fibres are carried out in another by small fibres, would explain the difficulty encountered by neuro-physiologists in attempting to exemplify their claim that the diameter of a fibre is an exact measure of its conduction velocity. The value placed on fibre diameter in formulae for determining the conduction velocity of a given fibre has varied a great deal from the day of Erlanger & Gasser's (1929) first classification of nerve fibres into A, B and C fibres. Grundfest (1940), however, indicates that the fast conducting A fibres range between the sizes of 20 and 1μ , which range of course includes almost all fibre sizes found in peripheral nerves. The data obtained for the determination of the physiological properties of nerve fibres having been gathered from animal nerves, it is to be assumed that of animals of the same species, the fibre composition of their nerves shows as much variation as in man. Investigators dealing with nerve conduction frequently related their findings to the fibre composition of the nerves they investigated, but counted only the large fibres and put in an

estimate for the small fibres. It is, however, this group which shows most noticeably individual variation. The interesting question arises, therefore, whether conduction velocity may not basically be related to the type of impulse carried as well as to the diameter. It is, for instance, well established that pain fibres are of the slow conducting type. Our own investigation is unable to give an answer to any of these questions, but explanations about the physiological functioning of nerve fibres will have to consider the morphological facts presented here. It has been experimentally established that in muscle nerves efferent and afferent fibres are contained in both the small and the large fibre groups (Eccles & Sherrington, 1930; Rexed & Therman, 1948; Drake & Stavraký, 1948).

An important problem requiring consideration here is the branching of nerve fibres. It is well established, that on reaching the muscles and other terminal organs nerve fibres branch considerably, but the amount of branching in the more proximal course of the nerve fibres, within the main nerve trunk and its muscular or cutaneous branches, is little known and considered to be not extensive. This is so stated by Eccles & Sherrington (1930) and Bjoerkman & Wohlfart (1936), and this whole problem has been recently surveyed by Sunderland & Lavarack (1951).

The problems discussed above, with relation to the muscular branches, are *mutatis mutandis* also applicable to the cutaneous nerves. For the cutaneous branches investigated the indices range from 2.9 to 11.5. Fig. 1 depicts the relation of the cutaneous nerve indices to those of the muscular branches. As outlined previously, this curve shows very well, how much larger, in each case, the number of small fibres is in the cutaneous branch. The range of variation again suggests that in certain persons with very few large fibres, functions must be carried out by small fibres. For instance, it is generally assumed that touch is conducted by large fibres only (Heinbecker, Bishop & O'Leary, 1933; Ranson, 1931; Erlanger & Gasser, 1929). If we do not assume that scarcity of large fibres in the cutaneous nerves of some persons indicates that the touch sensibility is quantitatively less represented, the only other conclusion is, that touch must be conducted in these subjects by small fibres also. Of course, the thought that obvious differences in sensitiveness between different people may be accounted for by such morphological differences in the composition of their cutaneous nerves is at least permissible.

The fibre composition of the sensory nerves in man was the subject of an extensive study by Ranson, Davenport, Droegemueller & Fisher (1934). In the cutaneous branches they investigated, a fibre composition similar to our own was found, but their graphs contain only the myelinated fibres and are not directly comparable to ours. We included myelinated and unmyelinated fibres together in our graphs. These authors give, however, ratios of myelinated to unmyelinated fibres, values similar to our index. Our indices, however, indicating how many times more small as compared with large fibres are present in a nerve, again contain within the number of small fibres some small myelinated as well as the unmyelinated fibres. This grouping together of unmyelinated and small myelinated fibres is consistent with the argument by Ranson, Davenport, Droegemueller & Fisher who, on comparative anatomical grounds, state that the two kinds of fibres are not essentially different. In support of this statement they quote an investigation by Duncan (1932) who showed that the preganglionic visceral efferent fibres in the ventral root of the

eighth thoracic nerve are to a large extent unmyelinated in the rat, mostly myelinated in the cat and entirely myelinated in the cow. Our own findings of course, as previously outlined, point in the same direction, as the individual variability in composition which we found suggests too that functions carried out in some individuals by large fibres must be carried out in others by small fibres when there are only a few large ones present. This individual variability incidentally was not considered by Ranson, Davenport, Droegemueller & Fisher in collecting their material, since the thirteen cutaneous nerves they investigated were from six different individuals. Although some of their conclusions in the direct comparison of these branches appear to us to be affected by the possibility of individual variation, such as their conclusion that there is a decrease in the number of unmyelinated fibres distally in the nerves of the upper extremity, their principal conclusions are unaffected by possible individual variations.

As for the muscular and cutaneous branches, analyses of the cross-sections of the popliteal nerves reflect the same individual variability of fibre composition found in the branches. A great variation exists also between the many bundles contained in the cross-section of one medial popliteal nerve. As outlined earlier, in one case all bundles within the cross-section of the nerve were analysed. It happened that this one was the nerve which contained the most small fibres, so many, in fact, that in some of its bundles hardly any large fibres were present. In all other cases we chose the bundle found to contain the most large fibres, the one containing the most small fibres and one intermediate in composition. The remaining bundles and their indices must vary then between the two extreme bundles. Fig. 2 shows the indices of the bundles investigated, plotted as in the case of the muscular and cutaneous branches, except that the intermediate bundle also is shown.

With the exception of the 56- and 78-year-old subjects, comparison of Figs. 1 and 2 shows that the index of the bundle with the greatest number of large fibres in the cross-section of the medial popliteal nerve has a lower index than the muscular branch investigated from the same nerve. Again, when we compare the indices of the bundles having the greatest number of small fibres with the indices of the cutaneous branches shown in the same curves, it is seen that the former are higher, except in the case of the 65-year-old subject where they are about equal. This relationship is an indication that in order to form a muscular or cutaneous branch out of these bundles, there has to take place an exchange of fibres to bring about the proportions found in the respective branches. This exchange of fibres adds small fibres to the bundles composed mainly of large ones, in order that it may become a muscular branch or vice versa for the formation of a cutaneous nerve. This exchange of fibres to bring about the right 'mixture' of the fibre groups is certainly one of the main purposes of the vast intraneural plexus. It is interesting to note, though not applicable in every case, that in most subjects the sum attained by adding the index of muscular branch and that of cutaneous branch is about equal to the sum of the indices of the bundle with most large fibres and the one with most small fibres. This near equality, which exists in all but the 56- and 72-year-old subjects, is striking enough when it is considered that in each bundle, by a statistical method, only 500 fibres were counted and measured, out of a considerably larger number of fibres. If one would undertake in a number of specimens to determine the

total number of fibres in each bundle (and in our experience this would be a vast undertaking) we are convinced the coincidence would be even closer. It would be interesting if we could have included in each subject the investigation of the fibre distribution of the anterior and posterior roots of the spinal cord of the segments contributing to the sciatic nerve. Such an investigation, however, was carried out on the roots of S_4 in the subject dealt with by Tomasch & Schwarzacher (1952). Although this segment does not participate in the formation of the sciatic nerve

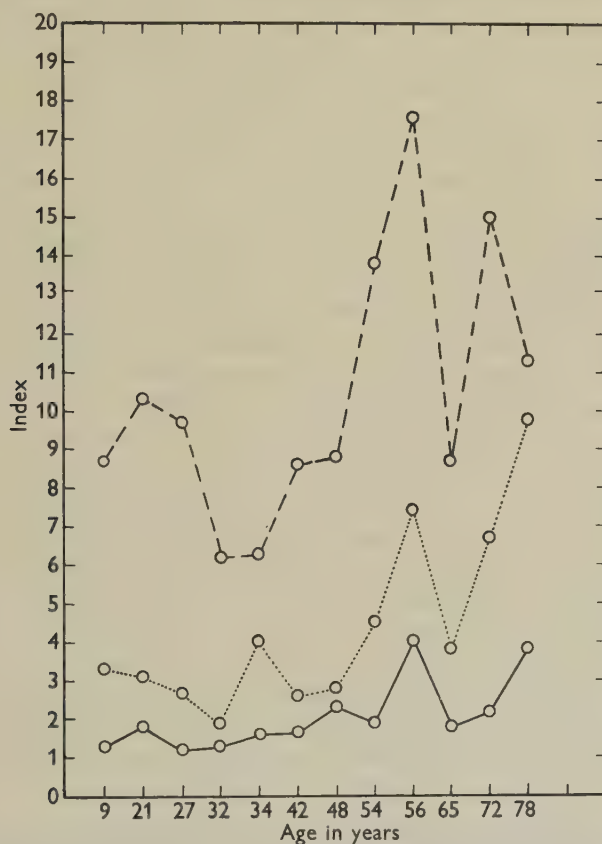


Fig. 2. Solid line, bundle of medial popliteal nerve containing fewest small fibres; dotted line, bundle of intermediate composition; interrupted line, bundle containing most small fibres.

the anterior root, with an index of 0.98, displays a composition similar to that of muscular branches in this individual and the posterior root, with an index of 2.03, resembles the composition of the cutaneous nerves of the same subject. Swensson (1938), who analysed the fibres of all anterior roots of one individual, showed for the roots forming the sciatic nerve a quite similar composition to S_4 . It can be assumed, therefore, that in this individual the index of this one segment must not have varied a great deal from those segments actually forming the sciatic. Gentile & Swensson (1941), who investigated all posterior roots in two individuals, show for the segments in question very similar composition to S_4 . It may be remarked here, that they did

not find great differences, but rather a great similarity between the two individuals they investigated. As we found a great variation in the fibre composition of peripheral nerves in a number of individuals, it remains to be seen whether roots are more uniform and changes occur in the further course of the nerve fibres to bring about such variations, or whether, when investigated together in one individual, roots and peripheral nerves will show close correlation in their composition. This similarity in fibre composition of posterior roots and cutaneous branches and of anterior roots and muscular branches must not, of course, lead to the assumption that they are distributed in the same proportion and represent the fibres of cutaneous and muscular branches. The whole process of mixing of the posterior and anterior root fibres in the spinal nerve and the consequent rearrangement of fibres by the intraneural plexus, permits each of the nerve branches to contain the necessary proportion of afferent and efferent fibres, that is to say, some derived from the anterior and some from the posterior root.

One more problem which may be considered is the comparison of the fibre sizes themselves in different nerves of one individual and between the several individuals investigated. In comparing the positions of the maxima and the minima in the cutaneous and muscular branches we found no fixed rule as to their relative positions. The maximum of the small fibres is most uniform; it lies with few exceptions at $1-2\mu$ in both muscular and cutaneous branches and in the bundles of the popliteal nerve of all individuals. The maximum of the large fibres, however, varies greatly. In some cases the muscular branch has this maximum a few microns higher than the cutaneous; in other subjects this maximum lies for both kinds of branches at the same level, and in still others the largest fibres in the cutaneous branch slightly exceed those in the muscular branch of the same subject. In comparing the different cases from this particular viewpoint our findings are as follows. The 21-year-old subject in the cutaneous branch has the characteristic points of the fibre-distribution curve at sizes $1-2\mu$ larger than in the muscular branch. However, the maximum of small fibres in the muscular branch is at $2-3\mu$ as compared with $1-2\mu$ in the cutaneous branch. The medial popliteal nerve itself has fibres generally larger than in the branches. In the 52-year-old subject we find this relation reversed. The fibres in the muscular branch are larger in almost all fibre classes. The bundle of the medial popliteal containing the greatest number of large fibres has its characteristic points at the same locations as the muscular branch. The bundle containing the greatest number of small fibres in the medial popliteal has larger fibres than the cutaneous branch. In the 42-year-old subject there is hardly any difference in the positions of the maxima and minima as between the muscular and cutaneous branches; the bundles of the medial popliteal contain fibres which are on the average 1μ larger than in the branches. In the 48-year-old individual the fibres in the motor branch are $1-2\mu$ larger than the fibres of the cutaneous branch and the fibre sizes in the bundles of the medial popliteal are similar to those in the branches. In the 54-year-old subject the fibres of the motor branch are $1-2\mu$ larger than in the cutaneous branch. The fibres in the bundles of the medial popliteal nerve are slightly bigger than in the branches.

In the 56-year-old subject the fibres in the muscular branch are again, on the whole, slightly larger than in the cutaneous branch, but the largest fibres in the

cutaneous nerve are 1μ larger. The fibres in the bundles of the medial popliteal are generally larger than in the branches. In the case of the 65-year-old individual the fibres of the muscular branch are 1μ smaller than in the cutaneous nerve. The fibres of the medial popliteal bundles are $2-3\mu$ larger than in the branches. In the 72-year-old subject the positions of the maxima and minima in the two kinds of branches are identical; however, the largest fibres in the cutaneous branch are $2-3\mu$ larger than in the muscular branch. The medial popliteal itself has fibres slightly bigger than in the branches. In the 78-year-old subject the fibres in the muscular branch are smaller than in the cutaneous branch, but in this case the largest fibres of the muscular branch are larger than in the cutaneous nerves. The fibres in the medial popliteal are again generally larger than in its branches.

In summing up the findings on this problem it can be said that so far as the position of the peak elevations of the large fibre group is concerned, between cutaneous and muscular branches of the same individual, there is no trend to the display of a significant difference. On the contrary, while in some individuals the fibres are in general slightly larger in the cutaneous nerves, in others the motor branch has the slightly larger fibres, and again in other subjects there is no difference at all. This is contrary to the findings of some authors dealing with animal nerves, where a tendency was described for the large fibres in the motor branches to be a few microns greater in size than the corresponding fibres in the cutaneous nerves of the same animal. Langley (1922), for instance, with reference to the cat, states: 'Thus the largest fibres whether of the anterior or of the posterior roots run to the striated muscle.'

To conclude, we may put forth the question as to the possible causes and the significance of the variability found in the nerve fibre composition of different individuals. When the work on this problem was started it was done with the view of gaining a better understanding of the two fibre groups known to exist in peripheral nerves and their significance; that such great variability would be found we did not expect, and since our investigation could not determine any causes for this variability the whole problem of the two fibre groups has become even more complicated. However, we were able to confirm that in all individuals there is a significant difference in the composition of limb muscle nerves and cutaneous nerves. This difference between motor and sensory nerves, which we also found in a previous investigation of the laryngeal nerve supply, is in our opinion the only constant clue as to the significance of the two fibre groups in the peripheral nerves. It points definitely towards a connexion of this problem with functional aspects. If it is proven that there is a principle governing the proportions of the two fibre groups in nerves of different function and that this is not incidental, then it seems safe to assume that the variation in the composition of these nerves in different individuals must be due to some other factor, about the nature of which, however, we have no information as yet. Of several probabilities considered in this matter we believe, in view of the wide range in the selection of our material, that age is not the determining factor. Fig. 1, for instance, shows such a fluctuation of the indices of both muscular and cutaneous branches that no relation to age is discernible. It remains to be investigated whether there may exist a relationship between the nerve-fibre composition in an individual and his body-type.

To determine this possibility it would be necessary to carry out an investigation similar to ours but to collect the nerves to be examined from subjects displaying the characteristic features of the different body-types, such as hyposthenic, asthenic, sthenic and hypersthenic individuals. Comparison of the extreme types would be of most value. Again it would be interesting to know whether an individual having reached maturity retains a constant composition as regards nerve-fibre population; whether there may exist a fluctuation caused by external factors such as the nutritional status, or whether this composition is inherited and remains unchanged. We believe that differences in the individual speed of reflexes or differences in the muscular tone and perhaps in sensitiveness may all have their basis in this variability. Some possible explanations as to the causes of this variation having been mentioned, it may be stated that in our opinion much of the individual variation may be explainable on the basis of differences in degree of myelination of the nerve fibres. However, it would be necessary to investigate the total number of fibres in several corresponding nerves of many individuals to determine if there may also exist a variability as to the total individual endowment of nerve fibres.

SUMMARY

A study was made of the fibre-calibre distribution of muscular and cutaneous branches of the sciatic nerve and cross-sections of the sciatic nerves themselves. The material selected represents all ages of human life. In one individual all the bundles of nerve fibres contained in the sciatic nerve were analysed, in twelve other cases the bundle containing the greatest number of large fibres and the bundle containing the largest number of small fibres and one of intermediate composition were chosen for investigation. Two principal findings emerged from these analyses.

(a) In each individual investigated, a cutaneous and muscular branch of the lower limb can be distinguished by the greater proportion of small fibres in the cutaneous nerve.

(b) Between different individuals there exists a great amount of variability in the composition of their nerves.

For expressing the relationship between the number of fibres of small size and number of large fibres in a nerve cross-section an index was found very useful. This index expresses how many times more fibres of small calibre are present as compared with the number of large fibres. In limb muscle nerves this index was found to vary from 0.9 to 4.7. For cutaneous nerves this variation is from 3.0 to 11.5. This means that, for example, when the index is 3.0, there are three times as many small fibres as large ones in the nerve cross-section investigated. No obvious correlation was found between the variability of nerve fibre composition and the age of the individuals. Other possible factors for this variation are considered. The variability found in the composition of nerves of different individuals necessitates the assumption that certain functions being carried out by fibres of large size in one individual, must be carried out by small fibres in another individual where the large fibres are very scarcely represented.

The authors wish to express their sincere thanks to the head of the Department, Prof. D. C. Matheson, for helpful assistance in the writing of this paper. The

investigation was carried out by aid of grants from the National Research Council of Canada and the William Spankie Memorial Fund of Queen's University, Kingston, Ontario.

REFERENCES

- ARNELL, N. (1936). Untersuchung über die Dicke des Achsenzylinders und der Markscheide in nicht fixierten Spinalnerven des Menschen und des Hundes, sowie über den Einfluss von Formalinfixierung, Paraffin-Einbettung und Ag-Imprägnierung auf dieselbe. *Acta psychiat. Kbh.*, **11**, 287-311.
- BJORKMAN, A. & WOHLFART, G. (1936). Faseranalyse der Nn. oculomotorius, trochlearis und abducens des Menschen und des N. abducens verschiedener Tiere. *Z. mikr.-anat. Forsch.* **39**, 631.
- DRAKE, CH. G. & STAVRAKY, G. W. (1948). An extension of the 'Law of Denervation to afferent neurons'. *J. Neurophysiol.* **11**, 229-238.
- DUNCAN, D. (1932). A determination of the number of unmyelinated fibres in the ventral roots of the rat, cat and rabbit. *J. comp. Neurol.* **4**, 459.
- ECCLES, J. C. & SHERRINGTON, C. S. (1930). Numbers and contraction values of individual motor-units examined in some muscles of the limb. *Proc. Roy. Soc. B*, **106**, 326-357.
- ERLANGER, J. & GASSER, H. S. (1929). The rôle of fibre size in the establishment of a nerve block by pressure or cocaine. *Amer. J. Physiol.* **88**, 581-591.
- FERNAND, S. V. & YOUNG, J. F. (1951). The sizes of the nerve fibres of muscle nerves. *Proc. Roy. Soc. B*, **139**, 38-58.
- GASSER, H. S. & ERLANGER, J. (1927). The role played by the sizes of the constituent fibres of a nerve-trunk in determining the form of its action potential wave. *Amer. J. Physiol.* **80**, 522.
- GENTELE, H. & SWENSSON, A. (1941). Über die Kaliberverhältnisse der hinteren Rückenmarkswurzeln beim Menschen. *Z. mikr.-anat. Forsch.* **50**, 190-206.
- GRUNDFEST, H. (1940). Bioelectric potentials. *Ann. Rev. Physiol.* **2**, 213-242.
- HÄGGQVIST, G. (1936). Analyse der Faserverteilung in einem Rückenmarksquerschnitt (T.II). *Z. mikr.-anat. Forsch.* **39**, 1-34.
- HEINBECKER, P., BISHOP, G. H. & O'LEARY, J. L. (1933). Pain and touch fibres in peripheral nerves. *Arch. Neurol. Psychiat.* **29**, 771-789.
- HJIANG, S. H. (1950). Über die Faserzahl und die Faserdicke in den Wurzeln des zweiten Thorakalnerven beim Menschen. *Acta anat.* **11**, 50-82.
- LANGLEY, J. N. (1922). The nerve fibre constitution of peripheral nerves and of nerve roots. *J. Physiol.* **56**, 382-396.
- RANSON, S. W. (1931). Cutaneous sensory fibres and sensory conduction. *Arch. Neurol. Psychiat.* **26**, 1122.
- RANSON, S. W., DAVENPORT, H. K., DROEGEMUELLER, W. H. & FISHER, C. (1934). Numbers, size and myelination of the sensory fibres in cerebrospinal nerves. *Res. Publ. Ass. nerv. ment. Dis.* **15**, 3-34.
- REXED, B. & THERMAN, P. O. (1948). Calibre spectra of motor and sensory nerve fibres to flexor and extensor muscles. *J. Neurophysiol.* **11**, 131-139.
- SÖGGQVIST, O. (1938). Studies on pain conduction in the trigeminal nerve. *Acta Psychiat. Kbh.*, **17** (suppl. 2), 1-130.
- SUNDERLAND, S. & LAVARACK, J. O. (1951). The branching of nerve fibres. *Acta anat.* **16**, 46-61.
- SWENSSON, Å. (1938). Über die Kaliberverhältnisse in den vorderen Rückenmarkswurzeln beim Menschen. *Z. mikr.-anat. Forsch.* **44**, 187-206.
- TOMASCH, J. & BRITTON, A. W. (1955). A fibre-analysis of the laryngeal nerve supply in man. *Acta anat.* **23**, 386-398.
- TOMASCH, J. & SCHWARZACHER, H. G. (1952). Die innere Struktur peripherer menschlicher Nerven im Lichte faseranalytischer Untersuchungen. *Acta anat.* **16**, 315-354.

DEGENERATION IN THE POST-COMMISSURAL FORNIX AND THE MAMILLARY PEDUNCLE OF THE RAT

By R. W. GUILLERY

Department of Anatomy, University College, London

INTRODUCTION

Gudden first showed that the mamillary region can be divided into a medial and a lateral nucleus, and claimed that the medial nucleus can be subdivided in terms of its connexions with the mamillo-thalamic and mamillo-tegmental tracts (Gudden, 1889). The mamillary region has since been subdivided in a variety of ways, and the topographical organization of the mamillary connexions has been studied by a variety of methods. Rose & Woolsey (1948) have shown that each of the anterior thalamic nuclei projects to one of the areas of the cingulate cortex, and Powell & Cowan (1954) and Cowan & Powell (1954) have subdivided the medial mamillary nucleus of the rabbit and the rat into three elements, each projecting to one of the anterior thalamic nuclei. The degeneration experiments of Sprague & Meyer (1950) suggest that the fornix ends in only two of these elements in the rabbit, but Simpson (1952) found fornix endings throughout the medial mamillary nucleus of the monkey. Tello, working on normal rat material (Tello, 1936-7), has divided the medial mamillary nucleus into an anterior and a posterior part, the former receiving mamillary peduncle fibres and the latter receiving fornix fibres. It is clear that there is a high degree of topographical organization of connexions in the mamillary system, and it is probable that the patterns of excitation reaching the parts of the cingulate cortex differ from each other and from that reaching the mid-brain via the mamillo-tegmental tract, but the precise organization of this system is not known at present.

The two afferent mamillary tracts, the post-commissural fornix and the mamillary peduncle also send fibres to other parts of the brain. Cajal (1911) has described fibres passing from the mamillary peduncle into the posterior hypothalamus, and a number of workers have described fibre bundles which leave the post-commissural fornix before this reaches the mamillary bodies (e.g. Gudden, 1889; Edinger & Wallenberg, 1902; Gerebtzoff, 1941-2). Guillery (1955) has shown that between a third and a half of the post-commissural fornix fibres fail to reach the mamillary bodies, and a hypothalamic ending for these fibres was suggested. The present degeneration studies were undertaken to trace the course of the non-mamillary fibres in the fornix and mamillary peduncle and to map out the mamillary endings of each of these tracts in relation to what is known of the origin of the mamillo-thalamic and mamillo-tegmental tracts.

MATERIALS AND METHODS

Eight rat brains with lesions in the hippocampal formation, two with lesions in the cortex and corpus callosum and four with lesions in the mid-brain were used. The hippocampal lesions were made with a small knife after the overlying cortex and

corpus callosum had been removed with a sucker. The mid-brain lesions were made by inserting a small, curved iridectomy knife under the cerebellum and into the floor of the fourth ventricle. The animals were allowed to survive 3–7 days, and were then killed by perfusion with 10 % formol saline. Some of the brains (see Table 1) were embedded in polyethylene glycol 1000MP followed by Nonex 63 (carbowax) and some were embedded in paraffin wax. The sections were stained by the method of Nauta & Gygas (1954).

Carbowax embedding only slightly alters the reaction of the Nauta and Gygas stain, the suppression of normal fibres being slightly more difficult, and usually more uneven, than on frozen sections. The method does not stain the paraffin embedded sections, but it has been possible to stain degenerating fibres and to suppress the staining of normal fibres by modifying the method slightly. In stage 5 of the published method a 0.05 % aqueous solution of phosphomolybdic acid was used for 10–15 min. In stage 6 a 0.01 % aqueous solution of potassium permanganate was

Table 1. *Summary of experimental material*

(Where two entries are separated by an oblique stroke the two halves of the brain have been treated separately. The side of the lesion precedes the oblique stroke.)

Animal no.	Post-operative survival (days)	Method of embedding	Plane of section	Lesion
195	7	Carbowax/not stained successfully	Parasagittal/not stained successfully	Hippocampus
212	3	Carbowax/carbowax	Parasagittal/horizontal	Hippocampus
213	5	Carbowax/carbowax	Parasagittal/horizontal	Hippocampus
214	7	Paraffin/carbowax	Frontal/horizontal	Hippocampus
216	3	Carbowax	Horizontal	Hippocampus
219	3	Carbowax	Horizontal	Mid-brain
220	4	Carbowax	Horizontal	Mid-brain
229	7	Paraffin	Frontal	Hippocampus
230	3	Paraffin	Frontal	Hippocampus
231	3	Paraffin	Frontal	Mid-brain
232	7	Paraffin	Parasagittal	Mid-brain
254	7	Carbowax	Frontal	Hippocampus
257	6	Carbowax	Frontal	Cortex
260	3	Carbowax	Frontal	Cortex

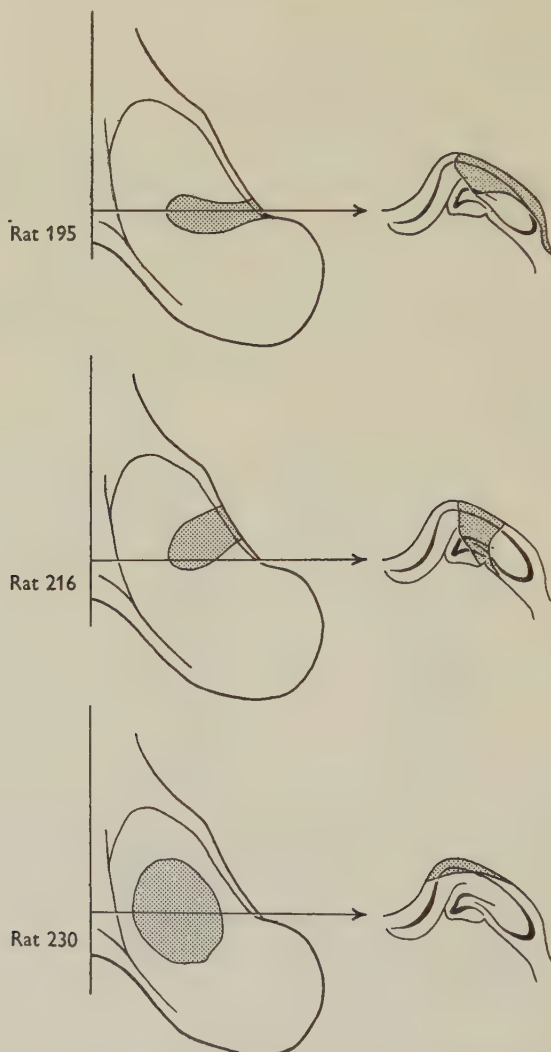
used for 3–9 min. The exact times, as in the original method, depend upon the tissue and upon the thickness of the sections. It is probable that this reduction in time and concentration is necessary because dehydration and clearing have removed fats which otherwise retard the action of the phosphomolybdic acid and potassium permanganate. Some sections have been stained by the Glees silver method for degenerating fibres (Glees, 1946), but since the Nauta and Gygas stain invariably produced a better result the majority of the sections were stained by the latter method.

Each group of sections stained by the silver methods was accompanied by a group of control sections from a normal animal which had been fixed and embedded in the same way as the operated material. These sections were taken through the same solutions as the experimental sections and, where the sections have to be treated individually, the normal and experimental material was alternated. Only those appearances that could be traced serially through the block to the lesion and could not be matched on the normal material have been accepted as degeneration.

RESULTS

(1) *Degeneration in the post-commissural fornix*

The lesions. Degeneration products have been found in the post-commissural fornix of all the animals in which the lesion includes the hippocampal formation (rats 195, 212, 213, 214, 216, 229, 230 and 254), but not in any of the other animals.

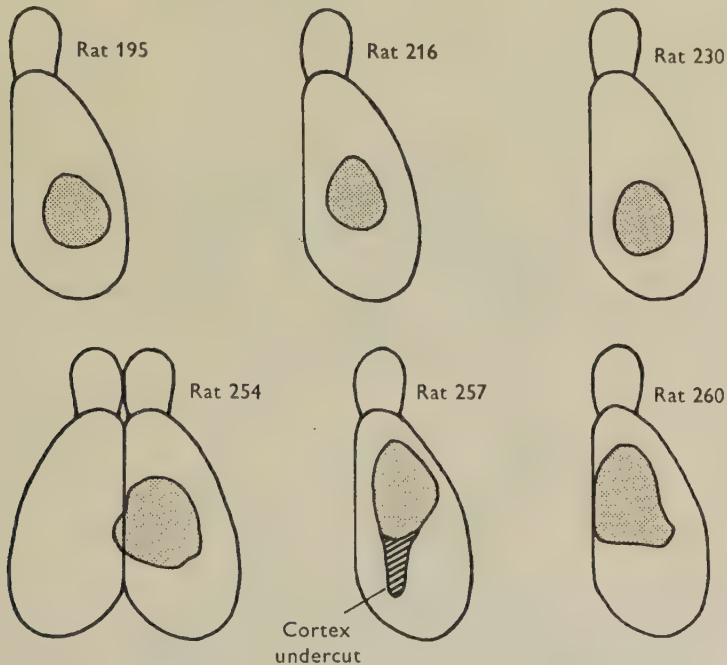


Text-fig. 1. The hippocampal lesions in rats 195, 216 and 230. Left: the dorsal aspect of the hippocampal formation. Right: frontal section in the plane of the arrows. The lesions are shown by stippling.

In each of the animals of the first group the lesion includes the dorsal part of the hippocampal formation, the corpus callosum and the overlying cortex. Three of the smaller lesions are shown in Text-fig. 1 (rats 195, 216 and 230). The minimal hippocampal lesion was found in *rat 230*, where a small part of the dorsal fold of the cornu

ammonis is damaged. In *rat 195* the lesion extends farther ventrally to include more of the cornu ammonis, a small part of the dentate fascia and the anterior portion of the fimbria. In *rat 216* the lesion is slightly larger than in *rat 195*, including the ventral fold of the cornu ammonis and extending farther posteriorly into the anterior part of the homolateral superior colliculus.

The lesions in *rats 213 and 229* resemble that of *rat 216* but spare the superior colliculus. In *rat 229* the dentate fascia has also been spared, but anteriorly the most dorsal part of the head of the caudate nucleus has been damaged. In *rats 212 and 214* the lesion is more extensive, including most of the antero-medial half of the hippocampal formation, and only sparing its most medial part in *rat 212*. Ventrally

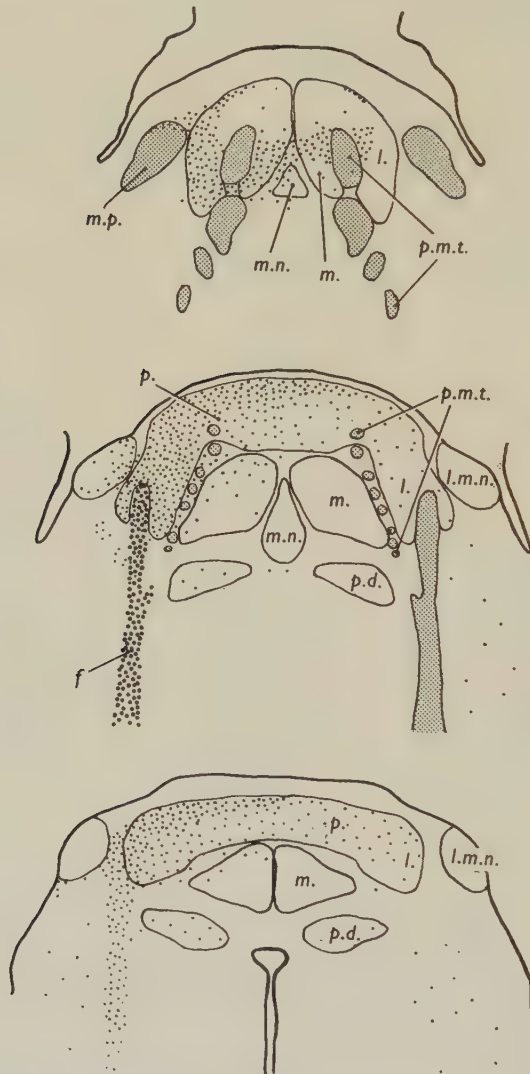


Text-fig. 2. The cortical lesions in rats 195, 216, 230, 254, 257 and 260.

there is extensive involvement of the homolateral thalamus, which includes the anterior nuclei, the lateral nucleus, the habenular nuclei and a part of the ventral nucleus in each animal. In *rat 254* the lesion lies in the anterior part of the hippocampal formation near the mid-line, including the hippocampal commissure, the anterior part of the fimbria and dorsal fornix, parts of the lateral septal nucleus, the head of the caudate nucleus, the antero-dorsal and antero-ventral thalamic nuclei, the lateral thalamic nucleus and the lateral habenular nucleus on the right. On the left the antero-medial part of the dorsal fornix and the most anterior parts of the cornu ammonis and dentate fascia have been damaged. The thalamus has been spared on the left.

The extent of the cortical damage in rats 195, 216, 230, 254, 257 and 260 is shown in Text-fig. 2.

The degeneration in the post-commissural fornix shows the same general pattern in all the animals. Degenerating fibres could be followed to the mamillary bodies, the anterior thalamus, the periventricular system and the rostral end of the mamillary peduncle, the degeneration being heaviest in the mamillary bodies, lighter and more



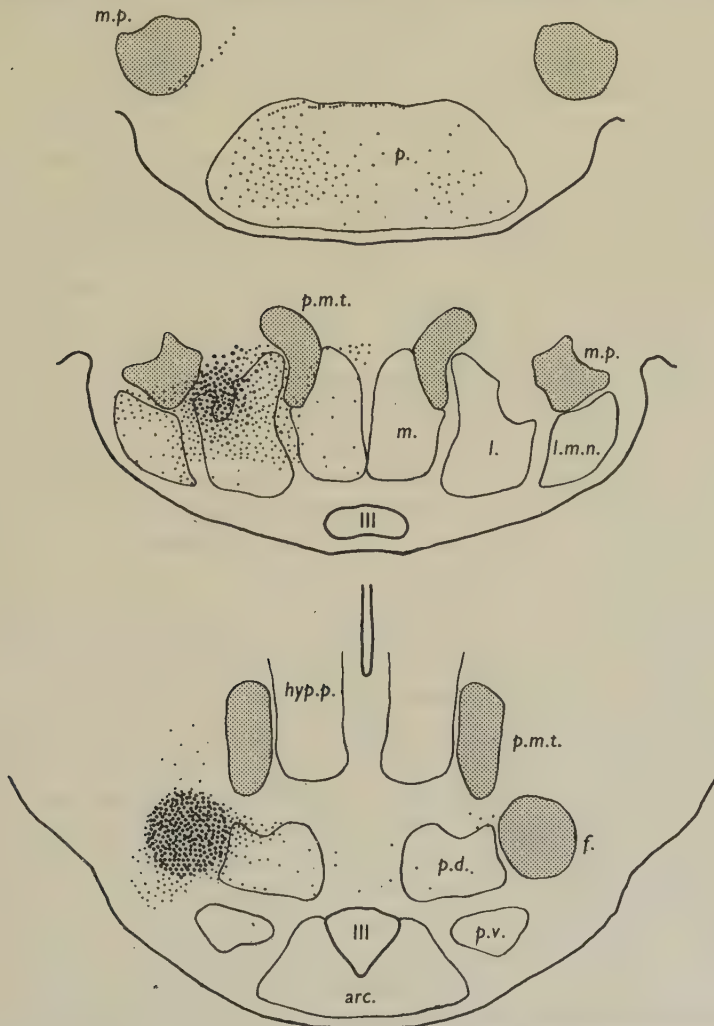
Text-fig. 3. The mamillary degeneration in rat 216. The three sections are cut in the horizontal plane; the most dorsal section lies at the top of the figure and the anterior part of each section lies towards the bottom of the figure.

variable in the anterior thalamus and sparse in the periventricular system and mamillary peduncle.

The fornix degeneration in the mamillary bodies. The distribution of the degeneration in the mamillary bodies of rats 216 and 229 is shown in Text-figs. 3 and 4.

Since the general pattern of the mamillary degeneration is the same in all the animals only one animal (rat 229) is described in detail below; minor differences are treated separately.

The fornix enters the mamillary region immediately lateral to the dorsal pre-mamillary nucleus,* sending a few fibres into this nucleus and a few across the mid-



Text-fig. 4. The mamillary degeneration in rat 229. The three sections are cut in the frontal plane; the most posterior section lies at the top of the figure.

line to its fellow. The main part of the fornix breaks up in the dorsal half of the pars lateralis, and from this a large number of fibres pass to the adjacent parts of the pars lateralis and into the pars posterior. Some of the fibres in the pars posterior curve medially and cross the mid-line into the opposite pars posterior. The homolateral

* The terminology of Gurdjian (1927) has been used for the nuclear groups in the mamillary bodies of the rat.

pars medialis receives a few scattered fibres from the adjacent pars lateralis and pars posterior. Two small fibre groups leave the fornix as it lies in the dorsal parts of the pars lateralis. One passes laterally into the lateral mamillary nucleus, where scattered degeneration products, heaviest in the dorsal and medial parts of the nucleus, can be seen. The second passes dorsomedially, its rostral portion forming a small cap over the pars medianus while the caudal portion enters the supra-mamillary nucleus and runs as small bundles towards the mamillo-tegmental tract. These bundles were lost in the region between the mamillary peduncle and the mamillo-tegmental tract.

Rats 195, 212, 213, 214, 216, 230 and 254 differ from rat 229 in the following features only. The dorsal premamillary nuclei of rats 213 and 195 are practically free of granules. The pars medianus is free of granules in rats 213 and 230; it shows a few doubtful granules in rat 195. The degeneration in the mamillary bodies of rat 216 is particularly heavy. The fibres that pass from the pars lateralis and the pars posterior into the pars medialis penetrate this element for a greater distance but the degeneration in the pars medialis is still relatively sparse. Contralaterally a number of degenerating fibres pass from the pars posterior into the pars lateralis and a few extend farther rostrally into the caudal parts of the medial forebrain bundle, immediately lateral to the fornix (Text-fig. 3). The contralateral pars lateralis and medial forebrain bundle of rats 212 and 214 also show some degeneration products, and in these two animals there is an appreciable amount of degeneration in the pars medialis, which is less dense than the degeneration in the rest of the medial nucleus and is coarser than the usual fornix degeneration, suggesting an origin from outside the fornix system.

A thalamic origin for these coarser fibres in the pars medialis is probable in view of the extensive thalamic involvement in these two animals. Le Gros Clark (1933) has described thalamo-mamillary degeneration in the rat travelling in the mamillo-thalamic tract from the anterior thalamic nuclei. Some coarse granules are present in the mamillo-thalamic tract of rat 212 but none was found in rat 214. On the basis of the present material it is not possible to make any definite statement about the precise origin of a possible thalamo-mamillary projection. However, if there is such a projection it may show the same topographical organization as the mamillo-thalamic projection since rat 254, in which only the antero-dorsal and antero-ventral thalamic nuclei have been damaged, shows no obvious coarse degeneration in the pars medialis, although there are degeneration granules in the mamillo-thalamic tract. In this animal the fornix degeneration is bilateral and the pars lateralis and pars posterior are packed with granules on both sides. The general pattern of the fornix distribution is the same as in rat 229.

Degeneration products have been found in the supra-mamillary region and the lateral mamillary nucleus of all the animals. In rat 216 it was not possible to identify the caudal component of the supramamillary group, and in rats 212, 214 and 254 a number of degenerating fibres of thalamic origin obscured the relationship of the supramamillary degeneration.

The mamillary distribution of the fornix can be summarized as follows. The post-commissural fornix ends largely in the homolateral pars lateralis and pars posterior of the medial mamillary nucleus, forming a considerable part of the pericellular

plexus of these elements. A number of fibres cross the mid-line in the posterior parts of the mamillary bodies and pass to the opposite pars posterior; from this a few fibres pass anteriorly into the opposite pars lateralis. The degeneration does not stop precisely at the boundaries of the mamillary elements, a few fibres entering the pars medialis, especially its posterior and lateral parts. These fibres can only form a minority of the total peri-cellular plexus of the pars medialis. Some fibres pass to the lateral mamillary nucleus, to the dorsal pre-mamillary nuclei and to the supra-mamillary nucleus. It is not certain that any of the last group end in the supra-mamillary nucleus.

Fornix degeneration in the anterior thalamic nuclei. As the fornix passes towards the posterior aspect of the anterior commissure it lies ventro-medial to the stria medullaris. In normal silver preparations a bundle of fibres can always be seen curving postero-dorsally from the fornix to the stria medullaris, the 'medial cortico-habenular tract'. In each of the rats in which there is fornix degeneration without thalamic involvement, and bilaterally in rat 254, this bundle is degenerating and can be followed from the dorsal aspect of the fornix to the posterior aspect of the stria medullaris. It does not join the stria medullaris but passes caudal to it, running laterally and slightly dorsally into the ventro-medial parts of the antero-ventral thalamic nucleus (Pl. 1, fig. 4a, b). This group of fibres has been called component A of the fornix in the following discussion.

Pl. 1, figs. 5a, b, show a second group of fibres that leaves the anterior parts of the post-commissural fornix. Rostrally these fibres are continuous with component A; they turn dorsally from the anterior third of the fornix to pass diffusely through the medio-ventral parts of the reticular nucleus and into the anterior and ventral parts of the antero-medial thalamic nucleus. They do not leave the fornix in a definite bundle, but form a scattered group of fibres immediately dorsal to the post-commissural fornix. Parasagittal sections show that a large number of these fibres turn through almost 180°, passing first dorsally for a short distance and then turning anteriorly towards the anterior pole of the antero-medial thalamic nucleus. A few of the fibres could be followed into the nucleus reuniens and rhomboideus and a few cross the mid-line into the opposite antero-medial thalamic nucleus. These fibres were found in rats 195, 213, 229 and bilaterally in rat 254. They could not be identified in rat 216, and there are only a few doubtful granules in this region in rat 230. This group has been called component B of the fornix in the following discussion.

Each of these two groups can be traced into its thalamic nucleus, but the precise thalamic distribution of the fornix has not been mapped because cortico-thalamic degeneration, entering the thalamus from its lateral aspect, obscures the relationships. This cortico-thalamic degeneration lies well lateral to the reticular nucleus in the anterior parts of the thalamus, but farther posteriorly it fills a large part of the reticular nucleus and passes into the ventral and lateral thalamic nuclei. It thus comes into close relation with the degeneration in the antero-ventral and the antero-medial nuclei. No distinct boundary could be seen in these regions, and there may be some overlap between the two groups of degeneration, particularly in the region of the reticular nucleus.

Although the present material does not show the precise distribution of the fibres that leave the dorsal aspect of the post-commissural fornix, it shows that a

considerable number of the fibres pass into the antero-ventral and antero-medial thalamic nuclei, and that few if any enter the antero-dorsal nucleus. The reticular nucleus may receive some fornix fibres and so may the nucleus rhomboideus and the nucleus reuniens.

Fornix degeneration in the periventricular system. A few fibres leave the fornix at the junction of its anterior and middle thirds. At their origin these fibres are continuous with component B, but they turn posteriorly rather than anteriorly and the two groups soon separate. The termination of these fibres has not been found. They pass medial to the mamillo-thalamic tract in the dorsal hypothalamus close to the third ventricle, following a course similar to the fibres that were shown leaving the cat's fornix on normal material (Guillery, 1955; Pl. 1, fig. 3). These fibres are shown most clearly in rats 195 and 213 where a few fibres could be traced into the mid-brain to be lost in the rostral part of the central grey. Rat 229 showed the rostral part of this group, but the fibres were soon lost on the frontal series. There is some doubtful degeneration in the periventricular region of rat 216, none in rat 230. The thalamic damage in the other three animals had caused considerable degeneration in the periventricular system, obscuring this component of the fornix completely. It is not possible to make accurate quantitative statements on the basis of degeneration appearances, but the present material suggests that only a small proportion of the fornix fibres enter this periventricular component (probably less than 5%).

The medial cortico-hypothalamic tract of Gurdjian (1927), passing from the anterior part of the post-commissural fornix into the periventricular region of the anterior hypothalamus, shows no degeneration in rats 195, 213, 216, and 230. It shows marked degeneration in rats 214 and 254, and slight degeneration in rats 212 and 229. The fibres in rats 214 and 254 could be followed caudally and ventrally, close to the ependyma of the third ventricle as far as the suprachiasmatic nucleus. They were lost either dorsal to or in this nucleus.

The rest of the hypothalamus has been carefully examined for degeneration granules in rats 195, 213, 216, 229 and 230. In none of these animals has it been possible to find certain evidence of degeneration in the hypothalamus apart from that described above. In view of the thalamic damage in rats 212, 214 and 254 these animals have been excluded from the hypothalamic investigation.

A hypothalamic ending for some of the fornix fibres cannot be completely excluded at present, but it is probable that the majority of the fibres that leave the post-commissural fornix pass to the anterior thalamus, and not to the hypothalamus as was previously suggested (Guillery, 1955).

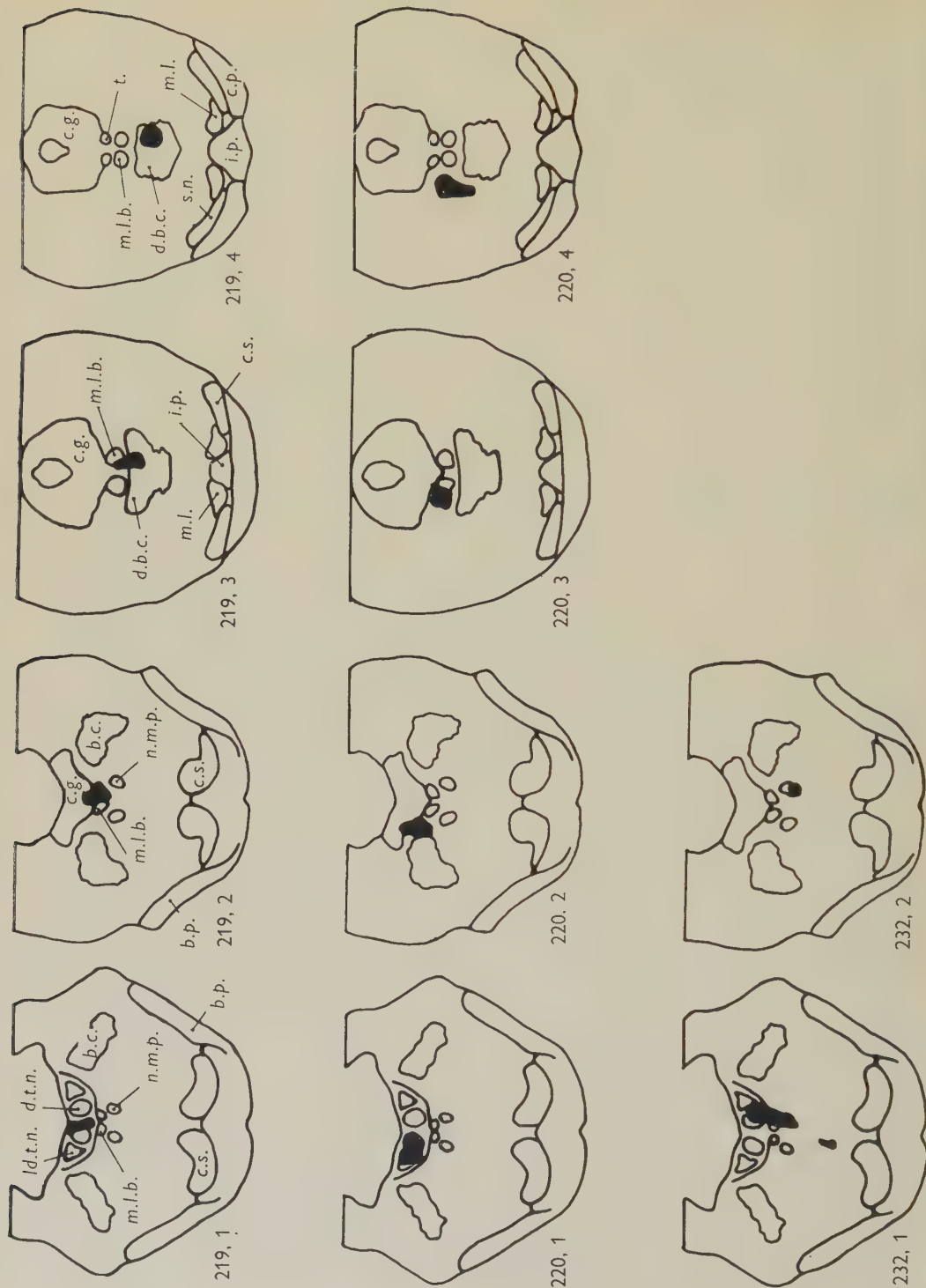
Fornix degeneration in the mamillary peduncle. A small number of degenerating fibres were followed from the fornix into the rostro-medial parts of the mamillary peduncle in all the animals. These fibres run in the mamillary peduncle for variable distances but most have left this tract at the exit of the third nerve. A few of these fibres could be seen passing dorso-medially from the rostral parts of the mamillary peduncle, the most rostral being continuous with the caudal part of the supra-mamillary component of the fornix.

(2) *Degeneration in the mamillary peduncle*

The lesions. The approximate extent of the lesions in rats 219, 220 and 232 is shown on frontal sections in Text-fig. 5. In all the animals of this group the knife entered the central grey through the floor of the fourth ventricle a short distance rostral to the genu of the seventh nerve. In *rat 220* the lesion is confined to the left side. The scar passes anteriorly, laterally and ventrally through the caudal parts of the central grey, including the whole of the dorsal tegmental nucleus (Gillilan, 1943), and a part of the latero-dorsal nucleus. It lies lateral to the medial longitudinal bundle and latero-dorsal to the deep tegmental nucleus (the nucleus medialis profundus of Gillilan). It ends at the level of the third nerve nucleus, lying in the reticular formation dorso-lateral to the decussation of the brachium conjunctivum. The dorso-caudal part of the lesion in *rat 219* lies close to the mid-line in the central grey between the genu of the seventh nerve and the dorsal tegmental nucleus. The lesion then divides into two. The caudal part passes ventrally into the reticular formation immediately rostral to the seventh nerve, while the rostral part includes the medial margin of the left dorsal tegmental nucleus and then deviates to the right to pass through the right medial longitudinal bundle and into the caudal portion of the decussation of the brachium conjunctivum on the right side of the mid-line. The right dorsal and both deep tegmental nuclei have been spared.

In *rat 232* the lesion again includes both sides of the brain but lies obliquely so that more rostral structures are damaged on the right than on the left. On the left the lesion passes through the central grey and medial longitudinal bundle into the reticular formation immediately rostral to the seventh nerve, well caudal to the dorsal and deep tegmental nuclei. On the right the lesion passes through the dorsal tegmental nucleus. It does not extend rostral or ventral to the deep tegmental nucleus. In *rat 231* there is a small scar in the latero-dorsal tegmental nucleus which lies immediately lateral to the dorsal tegmental nucleus but spares this entirely. The lesion does not leave the central grey.

The caudal course of the mamillary peduncle. Coarse degeneration products have been found in the mamillary peduncles of rats 219, 220 and 232. There are only a few doubtful granules in the mamillary peduncles of *rat 231*. In *rat 220* the right mamillary peduncle is free of degeneration while the left shows heavy degeneration; in *rat 219* there is light degeneration on the left and heavy degeneration on the right; *rat 232* shows degeneration on the right but not on the left. The caudal course of the degenerating fibres is similar in all three animals. The mamillary peduncle passes through the rootlets of the third nerve, lies lateral to the interpeduncular nucleus and then splits into two fibre groups. Both pass dorso-caudally in relation to the medial lemniscus. The larger lateral group passes round the lateral aspect of the medial lemniscus, where some of its fibres pass through the most medial part of the substantia nigra, while the medial group passes medial to the medial lemniscus lying between this and the interpeduncular nucleus. A few fibres pass through the medial lemniscus. All the fibres of the mamillary peduncle rejoin dorso-lateral to the medial lemniscus, where the mamillary peduncle fibres occupy their most lateral position. Farther caudally they become scattered and run dorso-medially caudal to the



Text-fig. 5. The mid-brain lesions in rats 219, 220 and 232. Note. The full caudal extent of the lesions has not been shown. In rat 220 the

decussation of the brachium conjunctivum, to become lost among other degenerating bundles.

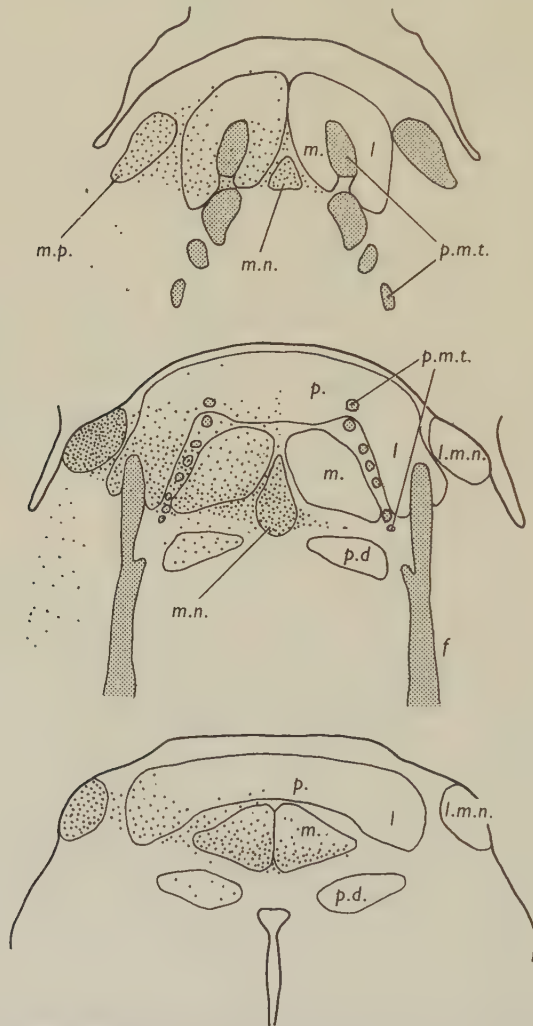
The direction of the caudal fibres suggests the deep or the dorsal tegmental nuclei as the most probable origin for the mamillary peduncle, and the distribution of the lesions supports this view. The course of the mamillary peduncle must be uncrossed since lesion and degeneration are confined to the left in rat 220. In rat 232 there is degeneration on the right side where the lesion includes the dorsal and the deep tegmental nuclei, but not on the left where the lesion passes caudal to these nuclei. In rat 219 the lesion damages the mamillary peduncle fibres on the right side as they lie caudal to the decussation of the brachium conjunctivum and damages the dorsal tegmental nucleus on the left side. The left deep tegmental nucleus has been spared in this animal, suggesting that at least some of the mamillary peduncle fibres arise from the dorsal tegmental nucleus. Rat 220 confirms this view since the dorsal tegmental nucleus is damaged but the deep tegmental nucleus spared, and rat 231, in which the dorsal tegmental nucleus has been spared, shows no degeneration in the mamillary peduncle. The normal dorsal tegmental nucleus has a relatively coarse pericellular plexus from which a bundle of coarse fibres passes ventrally and laterally, lateral to the medial longitudinal bundle (Pl. 1, fig. 6). This bundle most probably represents the dorsal tegmental origin of the mamillary peduncle. It is highly probable that other cell groups also give origin to mamillary peduncle fibres since normal mamillary peduncle fibres were seen in all the animals when the degenerating tracts were stained by the Glee's method.

The mamillary distribution of the mamillary peduncle. The distribution of the mamillary degeneration in rat 220 is shown in Text-fig. 6. The mamillary degeneration in rat 232 is lighter than that illustrated and the degeneration in rat 219 is slightly lighter but bilateral. Both animals show the same pattern of degeneration as rat 220. The density of the degeneration in the mamillary nuclei suggests that the individual fibres of the mamillary peduncle must branch and ramify extensively since the degeneration in the mamillary peduncle itself is sparse in comparison. The degeneration is heaviest in the lateral mamillary nucleus, but a dense group of degenerating fibres also passes medially through the pars lateralis of the medial mamillary nucleus into the anterior parts of the pars medialis and into the pars medianus. In the medial mamillary nucleus the degeneration becomes sparser as the pars posterior is approached, is light in the anterior half of the pars posterior and practically absent in the posterior half. A few fibres enter the dorsal pre-mamillary nucleus and a number cross the mid-line into the anterior parts of the opposite medial mamillary nucleus.

In the pars lateralis the majority of the fibres are directed towards the pars medianus; they do not form an irregular plexus such as is found in the pars medianus and the lateral mamillary nucleus. It is not possible to determine whether any fibres terminate in the pars lateralis, but the sections available suggest that this element may be a 'bed nucleus' for the mamillary peduncle fibres.

The degeneration in the medial forebrain bundle. Text-fig. 6 shows a number of fibres passing from the mamillary peduncle into the medial forebrain bundle. These can be followed rostrally in the ventro-lateral parts of the medial forebrain bundle as far as the diagonal band. Such fibres were also found in rats 232 and 219, but

the sections of rat 219 did not extend anteriorly beyond the pre-optic nucleus. These fibres appear to diminish in number as they are followed rostrally and a few of the fibres turn medially towards the medial hypothalamic nuclei. However, none of the animals showed convincing degeneration in the medial hypothalamus. All the fibres could be traced caudally into the mamillary peduncle.



Text-fig. 6. The mamillary degeneration in rat 220. The three sections are cut in the horizontal plane; the most dorsal section lies at the top of the figure and the anterior part of each section lies towards the bottom of the figure.

DISCUSSION

The degeneration that has been described in the previous sections has shown that the post-commissural fornix and the mamillary peduncle have a localized ending in the mamillary bodies and that each has a number of non-mamillary endings as well. These fibre groups form part of a complex system of connexions linking the

hippocampus, the mid-brain, the mamillary bodies, the hypothalamus, the anterior thalamus and the cingulate cortex. In the following discussion each fibre group has been considered separately in relation to the rest of this system of connexions.

The fornix degeneration in the mamillary bodies. Sprague & Meyer (1950) and Simpson (1952) have previously used a silver method to trace degeneration in the fornix. Other workers used the Marchi method and have given a variety of descriptions of the mamillary end of the fornix (e.g. Gerebtzoff, 1941-2; Allen, 1944; Morin, 1950). Although the Marchi method cannot give an accurate localization of non-myelinated endings, it is relevant to record that Edinger & Wallenberg (1902) traced Marchi degeneration to the ventro-lateral parts of the medial mamillary nucleus and to the lateral nucleus in the rabbit, while Vogt (1898*b*) followed Marchi degeneration to the posterior parts of the medial nucleus and to the lateral nucleus in the same species. These results agree closely with those of Sprague & Meyer, who, also working on the rabbit, found degeneration in the pars intermedialis and pars basalis of the medial mamillary nucleus (Rose's terminology; Rose, 1939). The present results show that in the rat there is also a localization of the majority of the fornix endings in the posterior and lateral parts of the medial mamillary nucleus and, together with the results of Sprague & Meyer they confirm Powell & Cowan (1954) who showed, from a study of the mamillo-thalamic projection, that the pars posterior and pars lateralis of the rat are together homologous to the pars intermedialis and the pars basalis of the rabbit.

Simpson found fornix degeneration throughout the medial mamillary nucleus of the macaque, which suggests that the organization of the primate mamillary system may differ considerably from that found in the rabbit and the rat (see below).

The mamillary peduncle degeneration in the mamillary bodies. Cajal (1911) traced the fibres of the mamillary peduncle into the medial and lateral mamillary nuclei on Golgi preparations. Probst (1902), Wallenberg (1899), Bodian (1940) and Fox (1941), among others, have traced ascending Marchi degeneration to the medial and lateral mamillary nuclei. Fox, working on the cat, and Wallenberg, working on the rabbit, have both described a localization of the mamillary peduncle degeneration in the anterior parts of the medial mamillary nucleus, and Tello (1936-7) has divided the medial mamillary nucleus into two parts, the anterior receiving its pericellular plexus from the mamillary peduncle and the posterior from the fornix. The degeneration of the mamillary peduncle in the rat cannot be strictly localized in Gurdjian's subdivisions of the mamillary region, but the distribution of this degeneration confirms the importance of the subdivisions. The lateral mamillary nucleus and the pars medianus of the medial mamillary nucleus receive the majority of the mamillary peduncle fibres, a considerable number of them pass to the pars medialis and many fibres pass through the pars lateralis. The pars posterior receives only a few mamillary peduncle fibres.

The fornix degeneration in the anterior thalamus. Most of the fibres in the anterior thalamic components of the fornix have been traced to the antero-ventral and antero-medial thalamic nuclei. Their origin has not been definitely established. In rat 254 only the medial cortex above the anterior end of the corpus callosum and the anterior part of the hippocampal formation are damaged on the left, and on this side both thalamic components show degeneration. The lesion in rat 260 includes

nearly the same part of the cortex but spares the hippocampal formation. Neither of the thalamic components of the fornix show any degeneration in this animal. The fibres that pass from the fornix to the anterior thalamus must therefore pass through the hippocampal formation, and a hippocampal origin appears most probable.

Vogt (1898*a*) described fibres from the post-commissural fornix passing into the anterior thalamus, and Cajal (1911) has shown the same group. It has not been possible to find a clear description of these fibres in any of the more recent accounts of the fornix. Vogt described the septum and not the hippocampus as the origin of these fornix fibres, but the present material, in which the septum has been spared in the majority of animals, shows that a considerable proportion of the anterior thalamic fibres do not come from the septum. Powell & Cowan (1955) have shown that at least a part of the 'medial cortico-habenular tract' arises from the septum, so that a second contribution to the anterior thalamus from the septum appears likely.

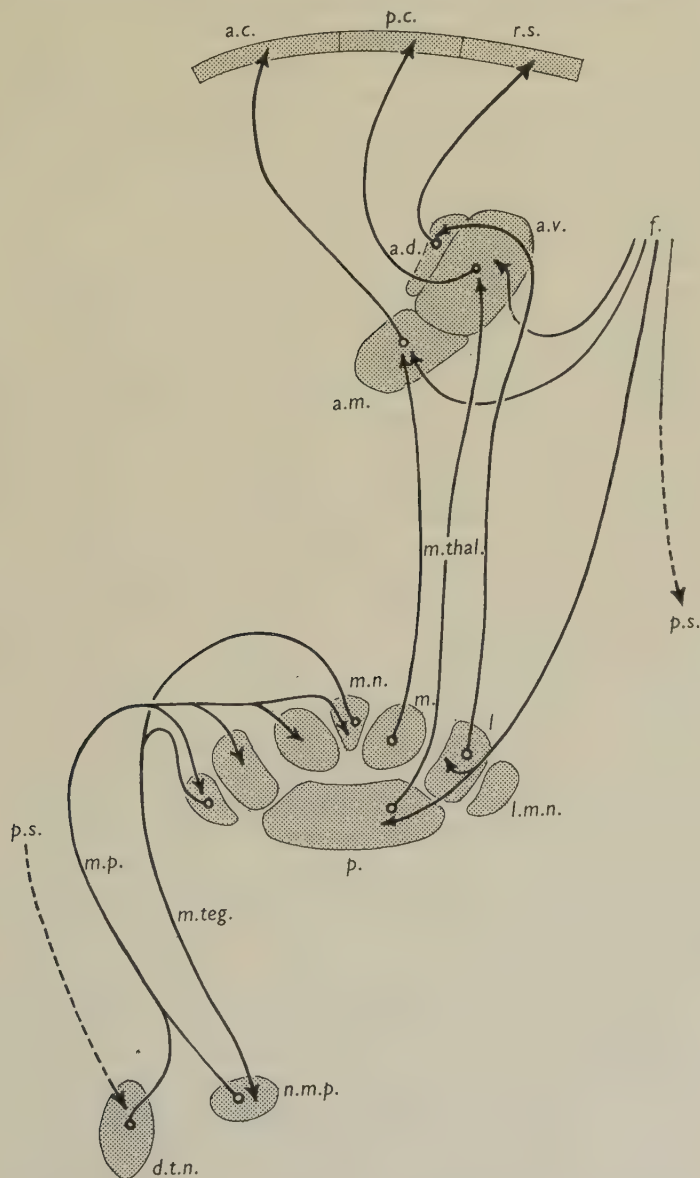
The organization of the mamillo-thalamic projection and of the anterior thalamic projection. Text-fig. 7 is based largely on the studies of Powell & Cowan (1954) and Cowan & Powell (1954) of the mamillo-thalamic projection, on Rose & Woolsey's (1948) investigation of the anterior thalamic projection and on the present results. It shows that each of the anterior thalamic nuclei, and thus each subdivision of the medial cortex, receives a different pattern of afferent impulses.

Stimulation and ablation experiments have shown that the anterior cingulate cortex is concerned with a wide variety of visceral and somatic responses (Kaada, 1951; Sloan & Kaada, 1953; Ward, 1948), and that it does not share these with other parts of the medial cortex. Text-fig. 7 shows that the anterior cingulate cortex can be influenced by mid-brain activity via the mamillary peduncle, and that this influence can be modified by impulses that travel in the thalamic fibres of the fornix. The posterior cingulate cortex appears to be more intimately linked to the hippocampus for it can be influenced by two types of fornix activity, one relaying in the mamillary bodies and anterior thalamus and the other relaying in the anterior thalamus only. The retrosplenial cortex only receives impulses that have crossed the mamillary synapse in the pars lateralis of the medial mamillary nucleus. Since the pars lateralis receives fibres from the fornix and the mamillary peduncle the retrosplenial cortex is in a position to receive a pattern of excitation that represents the total afferent discharge in the mamillary bodies at any one time.

Simpson's description of the mamillary end of the fornix suggests that in primates each of the three anterior thalamic nuclei receives fornix impulses that have been relayed in the mamillary bodies. Since the mamillary peduncle is relatively small in the monkey and in man, it is probable that the influence of mid-brain activity upon the cingulate cortex is reduced in primates.

The origin of the post-commissural fornix in the hippocampus. Daitz & Powell (1954) and Powell & Cowan (1955) have described a hippocampal localization of the origin of the parts of the fornix system. The present results confirm some of their findings in showing that the mamillary fibres of the fornix come from the dorsal fold of the alveus via the dorsal fornix and in showing that the medial cortico-hypothalamic tract travels in the fimbria. Rat 230 also suggests that component A travels in the dorsal fornix, while component B travels in the fimbria, but this needs

further confirmation. It is interesting to note that the CA₁ field of the cornu ammonis is related to the limbic cortex, not only by its connexions via the mamillary bodies but also by its original position in the dorsal part of the hippocampus.



Text-fig. 7. A diagrammatic representation of the mamillary connexions of the rat. See text.

The origin of the mamillary peduncle. Cajal (1911) described a double origin for the mamillary peduncle. The larger part comes from the region postero-inferior to the decussation of the brachium conjunctivum and crosses the medial lemniscus at right angles. The smaller comes from the medial lemniscus. Wallenberg (1899) had

also described a contribution from the medial lemniscus, but later investigations suggested that the mamillary peduncle receives no fibres from this tract (e.g. Probst, 1902; Ranson & Ingram, 1932). Retrograde cell changes have been observed in the deep tegmental nucleus after interruption of the mamillary peduncle (Quensel, 1911; Fox, 1941), and an origin in the nucleus of the mamillary peduncle has also been suggested by Papez (1932) and Fox (1941). The present material shows that some of the fibres of the mamillary peduncle must arise from the dorsal tegmental nucleus. A multiple origin from the dorsal and deep tegmental nuclei and perhaps from the nucleus of the mamillary peduncle is probable, but connexions with the medial lemniscus and the substantia nigra are best regarded as fibres of passage, not as an additional origin.

The afferent connexions of the dorsal and deep tegmental nuclei are not known at present. There is some evidence that the mamillo-tegmental tract ends in relation to the cells of the deep tegmental nucleus (Sanz, 1935; Morin, 1950), forming a circuit through the lateral mamillary nucleus and the pars medianus of the medial mamillary nucleus (see below). The deep tegmental nucleus lies medio-dorsal to the reticular formation of the mid-brain and immediately ventral to the medial longitudinal bundle. Its relation to the reticular formation and to the somatic motor pathways of the mid-brain remain to be determined. The dorsal tegmental nucleus lies in the central grey of the mid-brain and probably receives connexions from other parts of the periventricular system, particularly the hypothalamus (Crosby & Woodbourne, 1951). The fibres that pass from the fornix into the periventricular system may also establish an indirect link with the dorsal tegmental nucleus.

The mamillo-tegmental projection. The pars medianus of the medial mamillary nucleus and the lateral mamillary nucleus do not degenerate after anterior thalamic lesions (Powell & Cowan, 1954). The lateral mamillary nucleus sends its axons into the mamillo-tegmental tract (Sanz, 1935), and the pars medianus probably does so too (van Valkenburg, 1911). The mamillo-tegmental tract may send collateral branches into the mamillo-thalamic tract (Cajal, 1911; Tello, 1936-7), but since the precise thalamic distribution of these is not known at present they have not been included in Text-fig. 7. Text-fig. 7 shows that many of the impulses which reach the mamillary bodies via the mamillary peduncle are relayed back into the mid-brain, only some are relayed to the antero-medial and antero-dorsal thalamic nuclei via the mamillo-thalamic tract.

Two systems of interconnected neurons interact in the mamillary region, one including the hippocampus, the anterior thalamus and the medial cortex and the other including parts of the mid-brain. It is probable that closer study of the mid-brain connexions of the mamillary bodies will clarify the organization of the hippocampus and medial cortex, particularly that of the anterior cingulate region.

Other fibre groups. Small bundles of fornix fibres have been traced into the periventricular region of the anterior hypothalamus, the postero-dorsal hypothalamus and the mid-brain. Some fornix fibres have also been traced into the supramamillary region and the rostral part of the mamillary peduncle. These fibres form a relatively small part of the fornix, and most of them have been described previously (e.g. Vogt, 1898*b*; Edinger & Wallenberg, 1902; Gurdjian, 1927). It has not been possible to trace any of these fibres to a definite end-station, but a number must end in the

periventricular region of the hypothalamus and mid-brain, while others may pass into the reticular formation of the mid-brain.

The mamillary peduncle sends a number of its fibres into the medial forebrain bundle rostral to the mamillary bodies. These fibres have been described by Cajal (1911) and by Tello (1936-7), but have never been traced beyond the caudal parts of the hypothalamus. In the present material a number of fibres were followed rostrally through the lateral hypothalamus as far as the diagonal band. Green & Arduini (1954) have shown that a characteristic hippocampal response can be induced by stimulation of peripheral afferents or by stimulation of the mid-brain tegmentum, hypothalamus, pre-optic region and septum. The course of the mamillary peduncle fibres shows that they must be closely related to this ascending system, but their number suggests that they form only a small part of it.

General conclusions. The mamillary bodies form a part of a complex system of partially independent projections which link the mid-brain and the hippocampus to the medial cortex, and bring a characteristic pattern of excitation to each of the three major subdivisions of this cortex. This system of connexions has been represented diagrammatically in Text-fig. 7, but a number of components have been excluded. The pre-commissural fornix is not shown, and the complications that are introduced into the topographical organization by recurrent branches from the mamillo-thalamic axons (Cajal, 1911), by the spread of the dendritic plexus across the boundaries of the mamillary elements or by the overlap of the fornix and mamillary peduncle endings in the mamillary bodies have not been considered.

Le Gros Clark (1938) has described the mamillo-thalamic tract as a local differentiation in the hypothalamo-thalamic part of the periventricular system and the present results have again stressed the close relationship between the mamillary system of connexions and the periventricular system. By means of the periventricular fibres of the fornix the activity of the mamillary bodies can be brought into relation with that of other parts of the hypothalamus, the mid-line and medial nuclei of the thalamus and the central grey of the mid-brain. The organization of the periventricular system, however, must be studied in more detail before the nature of this relationship can be understood. A direct or indirect link between the mamillary bodies and the mid-brain reticular formation via the mamillary peduncle, the mamillo-tegmental tract and the supra-mamillary fibres of the fornix also appears highly probable.

The mamillary bodies can be regarded as an important relay in the specific afferent pathway to the medial cortex, having a number of connexions with the diffuse systems of the brain stem. A further analysis of these latter connexions may prove particularly useful to an understanding of the activity of the medial cortex.

Note. W. J. H. Nauta has recently studied degeneration in the fornix system of the rat, and has kindly sent me a copy of a manuscript now in the press with the *Journal of Comparative Neurology*. He has described a pattern of degeneration in the post-commissural fornix closely similar to that reported here. The major difference between his findings and the present ones concerns the mamillary ending of the fornix. He describes fornix fibres distributing throughout the mamillary region but concentrated mainly in the lateral parts of the medial mamillary nucleus.

SUMMARY

1. Degeneration in the post-commissural fornix and the mamillary peduncle of the rat has been studied by the silver method of Nauta & Gyax (1954).

2. Degenerating fornix fibres have been traced to the mamillary bodies, the anterior thalamus, the periventricular system and the rostral part of the mid-brain.

3. Mamillary peduncle fibres have been traced to the mamillary nuclei and into the medial forebrain bundle.

4. The majority of the mamillary fibres of the fornix pass into the pars posterior and the pars lateralis of the medial mamillary nucleus, most on the same side, some crossed.

5. The mamillary fibres of the mamillary peduncle pass to the pars medianus, the pars medialis and the pars lateralis of the medial mamillary nucleus and to the lateral mamillary nucleus; only a few of these cross in the anterior parts of the medial mamillary nucleus.

6. A large number of fibres leave the dorsal aspect of the post-commissural fornix and enter the antero-medial and antero-ventral thalamic nuclei. Few, if any, enter the antero-dorsal nucleus.

7. The organization of the mamillary system of connexions is such that each of the major fields of the medial cortex (anterior cingulate, posterior cingulate and retrosplenial) receives a different pattern of afferent impulses.

I wish to thank Prof. J. Z. Young for his encouragement and criticism, Miss B. Shirra for technical assistance and Mr J. Armstrong and Miss T. Marchmant for the photomicrography.

REFERENCES

- ALLEN, W. F. (1944). Degeneration in the dog's mamillary body and Ammon's horn following transection of the fornix. *J. comp. Neurol.* **80**, 283-291.
- BODIAN, D. (1940). Studies on the diencephalon of the virginia opossum. Part II, the fibre connections in normal and experimental material. *J. comp. Neurol.* **72**, 207-297.
- CLARK, W. E. LE GROS (1933). An experimental study of the thalamic connections in the rat. *Phil. Trans. B*, **222**, 1-28.
- CLARK, W. E. LE GROS (ed.) (1938). In *The Hypothalamus*. Edinburgh: Oliver and Boyd.
- COWAN, W. M. & POWELL, T. P. S. (1954). An experimental study of the relation between the medial mamillary nucleus and the cingulate cortex. *Proc. Roy. Soc. B*, **143**, 114-125.
- CROSBY, E. C. & WOODBOURNE, R. T. (1951). The mammalian midbrain and isthmus region. Part II, the fibre connections. C, hypothalamo-tegmental pathways. *J. comp. Neurol.* **94**, 1-32.
- DAITZ, H. M. & POWELL, T. P. S. (1954). Studies of the connections of the fornix system. *J. Neurol. Psychiat.* **17**, 75-82.
- EDINGER, L. & WALLENBERG, A. (1902). Untersuchungen ueber den Fornix und das Corpus mamillare. *Arch. Psychiat. Nervenkr.* **35**, 1-21.
- FOX, C. A. (1941). The mamillary peduncle and the ventral tegmental nucleus in the cat. *J. comp. Neurol.* **75**, 411-425.
- GEREBTZOFF, M. A. (1941-2). Notes anatomo-expérimentales sur le fornix, la corne d'ammon et leur relation avec diverses structures encéphaliques, notamment épiphysiques. *J. belg. Neurol.* **41/42**, 199-206.
- GILLILAN, L. A. (1943). The nuclear pattern of the non-tectal portions of the midbrain and isthmus in rodents. *J. comp. Neurol.* **78**, 213-251.

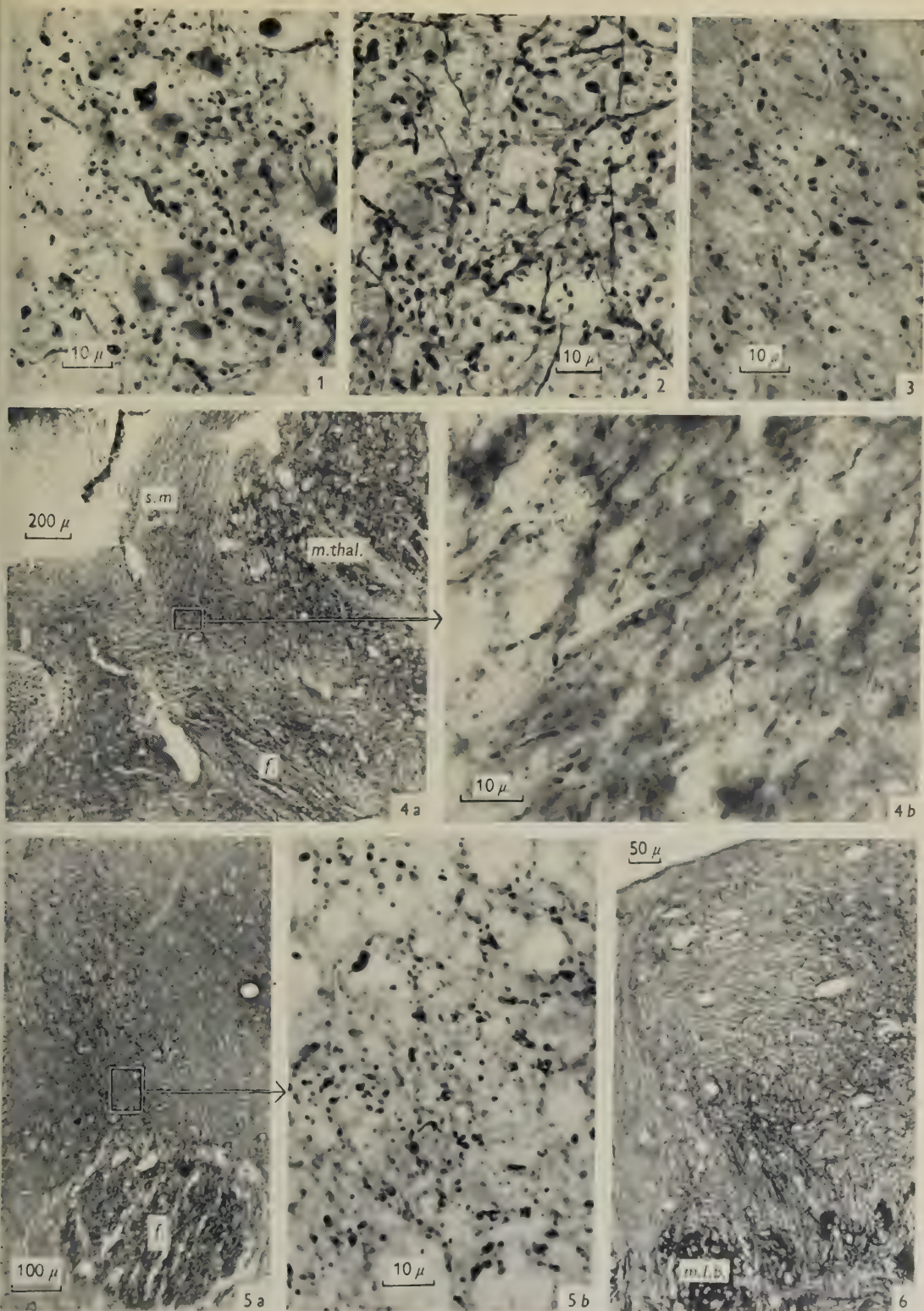
- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new method. *J. Neuropath. exp. Neurol.* **5**, 54-59.
- GREEN, J. D. & ARDUINI, A. A. (1954). Hippocampal electrical activity in arousal. *J. Neurophysiol.* **17**, 533-557.
- GUDDEN, B. VON (1889). *Gesammelte und hinterlassene Abhandlungen*. Wiesbaden; Bergmann.
- GUILLERY, R. W. (1955). A quantitative study of the mamillary bodies and their connexions. *J. Anat., Lond.*, **89**, 19-32.
- GURDJIAN, E. S. (1927). The diencephalon of the albino rat. *J. comp. Neurol.* **43**, 1-114.
- KAADA, B. R. (1951). Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of 'rhinencephalic' and other structures in primates, cat and dog. *Acta physiol. scand.* **24** (Suppl. 83), 1-285.
- MORIN, F. (1950). An experimental study of the hypothalamic connections in the guinea-pig. *J. comp. Neurol.* **92**, 193-213.
- NAUTA, W. J. H. & GYGAX, P. A. (1954). Silver impregnation of degenerating axons in the central nervous system: a modified technique. *Stain Tech.* **29**, 91-93.
- PAPEZ, J. W. (1932). The nucleus of the mamillary peduncle. *Anat. Rec.* **52**, 72-73.
- POWELL, T. P. S. & COWAN, W. M. (1954). The origin of the mamillo-thalamic tract in the rat. *J. Anat., Lond.*, **88**, 489-497.
- POWELL, T. P. S. & COWAN, W. M. (1955). An experimental study of the efferent connexions of the hippocampus. *Brain*, **78**, 115-132.
- PROBST, M. (1902). Experimentelle Untersuchungen ueber die Anatomie und Physiologie der Leitungsbahnen des Gehirnstammes. *Arch. Anat. Physiol., Lpz.* (Suppl. Anat.), Jg. 1902, pp. 147-254.
- QUENSEL, F. (1911). Untersuchungen ueber die Tektonik von Mittel-und Zwischenhirn des Kaninchens. *Pflug. Arch. ges. Physiol.* **139**, 47-92.
- RAMON Y CAJAL, S. (1911). *Histologie du système nerveux de l'homme et des vertébrés*, Vol. II. Paris: A. Maloine.
- RANSON, S. W. & INGRAM, W. R. (1932). The diencephalic course and termination of the medial lemniscus and brachium conjunctivum. *J. comp. Neurol.* **56**, 257-275.
- ROSE, J. (1939). The cell structure of the mamillary body in mammals and in man. *J. Anat., Lond.*, **74**, 91-115.
- ROSE, J. & WOOLSEY, C. N. (1948). Structure and relations of limbic cortex and anterior thalamic nuclei in rabbit and cat. *J. comp. Neurol.* **89**, 279-348.
- SANZ, IBANEZ, J. (1935). Étude de la dégénération du fascicule tegmental de Gudden consécutif à la lésion expérimentale du noyau mamillaire externe. *Trab. Lab. Invest. biol. Univ. Mad.* **30**, 211-219.
- SIMPSON, D. A. (1952). The efferent fibres of the hippocampus in the monkey. *J. Neurol. Psychiat.* **15**, 79-92.
- SLOAN, N. & KAADA, B. R. (1953). Effects of anterior limbic stimulation on somato-motor and electro-cortical activity. *J. Neurophysiol.* **16**, 203-220.
- SPRAGUE, J. M. & MEYER, M. (1950). An experimental study of the fornix of the rabbit. *J. Anat., Lond.*, **84**, 354-368.
- TELLO, J. F. (1936-7). Évolution, structure et connexions du corps mamillaire chez la souris blanche, avec des indications chez d'autres mammifères. *Trab. Lab. Invest. biol. Univ. Mad.* **31**, 77-142.
- VALKENBURG, C. T. VAN (1911). Caudal connections of the corpus mamillare. *Proc. Acad. Sci. Amst.* **14**, II, 1118-1121.
- VOGT, M. O. (1898a). Sur un faisceau septo-thalamique. *C.R. Soc. Biol., Paris* (Series 10), **5**, 206-207.
- VOGT, M. O. (1898b). Sur le pilier antérieur du trigone. *C.R. Soc. Biol., Paris* (Series 10), **5**, 207-208.
- WALLENBERG, A. (1899). Notiz über einen Schleifenursprung des Pedunculus corporis mamillariss beim Kaninchen. *Anat. Anz.* **16**, 156-158.
- WARD, A. A. (1948). The cingular gyrus: area 24. *J. Neurophysiol.* **11**, 13-23.

LIST OF ABBREVIATIONS

<i>a.c.</i>	anterior cingulate cortex	<i>m.lb.</i>	medial longitudinal bundle
<i>a.d.</i>	antero-dorsal nucleus of the thalamus	<i>m.n.</i>	pars medianus of the medial mamillary nucleus
<i>arc.</i>	arcuate nucleus of the hypothalamus	<i>m.p.</i>	mamillary peduncle
<i>a.m.</i>	antero-medial nucleus of the thalamus	<i>m.teg.</i>	mamillo-tegmental tract
<i>a.v.</i>	antero-ventral nucleus of the thalamus	<i>m.thal.</i>	mamillo-thalamic tract
<i>b.c.</i>	brachium conjunctivum	<i>n.m.p.</i>	nucleus medialis profundus (deep tegmental nucleus)
<i>b.p.</i>	brachium pontis	<i>p.</i>	pars posterior of the medial mamillary nucleus
<i>c.g.</i>	central grey	<i>p.c.</i>	posterior cingulate cortex
<i>c.p.</i>	cerebral peduncle	<i>p.d.</i>	dorsal premamillary nucleus
<i>c.s.</i>	cerebro-spinal tract	<i>p.m.t.</i>	principal mamillary tract (the common origin of the mamillo-thalamic and mamillo-tegmental tracts)
<i>d.b.c.</i>	decussation of the brachium conjunctivum	<i>p.s.</i>	periventricular system
<i>d.t.n.</i>	dorsal tegmental nucleus	<i>p.v.</i>	ventral premamillary nucleus
<i>f.</i>	fornix	<i>r.s.</i>	retrosplenial cortex
<i>hyp.p.</i>	posterior hypothalamic nucleus	<i>s.m.</i>	stria medullaris
<i>i.p.</i>	interpeduncular nucleus	<i>s.n.</i>	substantia nigra
<i>l.</i>	pars lateralis of the medial mamillary nucleus	<i>t.</i>	trochlear nucleus
<i>ld.t.n.</i>	laterodorsal tegmental nucleus	<i>III</i>	third ventricle
<i>l.m.n.</i>	lateral mamillary nucleus		
<i>m.</i>	pars medialis of the medial mamillary nucleus		
<i>m.l.</i>	medial lemniscus		

EXPLANATION OF PLATE

- Fig. 1. Fornix degeneration in the pars posterior of the medial mamillary nucleus. Rat 216. Nauta and Gyax method.
- Fig. 2. Mamillary peduncle degeneration in the pars medialis of the medial mamillary nucleus. Rat 220. Nauta and Gyax method.
- Fig. 3. Fornix degeneration approaching the anterior pole of the antero-medial thalamic nucleus. Rat 229. Modified Nauta and Gyax method.
- Fig. 4*a, b*. Degeneration in component A of the fornix ascending behind the stria medullaris. Parasagittal section. Rat 195. Nauta and Gyax method.
- Fig. 5*a, b*. Degeneration in component B of the fornix; a large scattered group of degenerating fibres is leaving the dorsal aspect of the fornix. Frontal section. Rat 229. Modified Nauta and Gyax method.
- Fig. 6. The dorsal tegmental nucleus of the rat. Note the bundle of coarse fibres leaving the ventral aspect of the nucleus. Bielschowsky method.





EXPERIMENTAL STUDIES OF THE VERMAL CEREBELLAR PROJECTIONS IN THE BRAIN STEM OF THE CAT (FASTIGIOBULBAR TRACT)

BY DONALD M. THOMAS, RONALD P. KAUFMAN, JAMES M. SPRAGUE
AND WILLIAM W. CHAMBERS

*Department of Anatomy and Institute of Neurological Sciences School
of Medicine, University of Pennsylvania, Philadelphia*

INTRODUCTION

The anatomical studies of Jansen & Brodal (1940, 1942) proposed a new concept for the morphological organization of the cerebellum, based on the efferent relations between the cerebellar cortex and the deep cerebellar nuclei of rabbit, cat and monkey. They found the cerebellum organized into three non-overlapping longitudinal zones: (1) the vermal, consisting of the most medial cortex and fastigial nuclei, (2) the paravermal, consisting of intermediate cortex and interposed nuclei, and (3) the lateral, hemispheric cortex and dentate nuclei (Text-fig. 1). These anatomical subdivisions were shown to have functional validity in the cat by the work of Sprague & Chambers (1953, 1954) and Chambers & Sprague (1955*a, b*). These authors showed that the syndrome following lesions of the paravermal-lateral zones is indistinguishable from that following pyramidal section in this animal, whereas the contribution of the vermal zone to motor function is markedly different and clearly extrapyramidal. Moreover, Chambers (1947), Moruzzi (1950), Zanchetti & Zoccolini (1954), and others, have shown that this medial zone is capable of exerting autonomic as well as somatic functions in the cat. This concept of longitudinal, corticonuclear organization of the cerebellum is at variance with the widely accepted lobular theory of Ingvar (1923), Larsell (1937), Fulton & Dow (1937) and Dow (1942). This new concept and the data supporting it, have been set forth and discussed in detail by Chambers & Sprague (1955*a, b*).

The anatomical and functional identification of a longitudinal, corticonuclear, zonal organization in the cerebellum makes pertinent an attempt to learn whether the efferent cerebellar nuclear projections to the brain stem are equally distinct. The literature is equivocal on this point. The vermal zone, whose functional characteristics have already been shown to be similar to those of the medial, medullary reticular formation (Sprague & Chambers, 1954), forms the basis of the present report. Another study of the brain stem projection of the paravermal and lateral zones (interposed and dentate nuclei) is in preparation (Cohen, Chambers & Sprague, 1956).

Allen (1924) has reviewed the early studies of the efferent projections of the cerebellar nuclei. From his own work on the guinea-pig, he believed that the medial nucleus (fastigius) and the lateral nuclei (interpositus and dentate) project to different areas of the brain stem reticular formation, whose function has been the

subject of so much recent study in cat and monkey. Study of specific cerebellar nuclear lesions in the cat has been previously attempted only by Mussen (1927) and Rasmussen (1933). Both of these studies employed the Marchi technique. The present study was made with a silver technique (Nauta & Gygyax, 1954) which stains selectively degenerating axons, and thus allows the tracing not only of the fibre tracts but of the unmyelinated, terminal fields of these tracts.



Text-fig. 1. Schematic drawing of the cerebellar cortex (left) and nuclei (right) of the cat (Jansen & Brodal, 1940, courtesy of the Wistar Press), showing the longitudinal corticonuclear zones. The extent of the fastigial lesion is shown on the left of the nuclear picture.

The results of the present study confirm previous findings (see Rasmussen, 1933; Rand, 1954) on the major pathways of the fastigiobulbar tracts lying in the restiform body and the brachium conjunctivum. In addition, the silver method used here reveals many new details of the nuclear termination of the paths which reach from the rostral thalamus to the upper cervical spinal cord. The fastigial projection is bilateral to the brain stem and cord, the decussation having occurred in the cerebellum. No degeneration was found in somatic, cranial or spinal motor nuclei, or in other cerebellar nuclei except the opposite fastigius. The degeneration was found throughout the reticular formation in medulla, pons, mesencephalon and thalamus (mid-line, intralaminar) and in the intermediate grey of the spinal cord; everywhere this projection was primarily contralateral and medial. Only in the vestibular nuclei (lateral, superior) was the degeneration not symmetrical in distribution, and in addition it was here primarily ipsilateral to the lesion.

METHODS

This report is based on a series of five adult cats. Unilateral lesions of the fastigial nucleus were made electrolytically with the aid of the Johnson stereotaxic instrument. The monopolar nichrome electrode (0.0036 in.) was passed into the posterior cerebellum at 30–90° from the vertical in order to minimize damage to the cortex overlying the lesion.

Pre- and postoperatively, the animals were examined for posture, tone, placing, hopping and other reflexes, and for gait both on the ground and on elevated horizontal bars. This physiological information has been published in detail elsewhere (Chambers & Sprague, 1955*a, b*). After a survival period of 4–7 days, the animals were sacrificed by intravascular perfusion with formol-saline-acacia according to the method of Koenig, Groat & Windle (1945). The brains were removed and hardened in 10% formol-saline for 1–2 weeks.

The upper cervical cord, brain stem and cerebellum were cut into frozen sections of 20 μ . Satisfactory freezing temperature was maintained by use of a mixture of dry ice and butyl alcohol applied to a copper stage and placed in a sliding microtome. Groups of ten consecutive sections were assembled in small dishes, and from each dish, representing $\frac{1}{3}$ mm. of tissue, two to four representative sections were taken for staining by the silver degeneration technique, and two sections for staining with thionin. The silver degeneration method described by Nauta & Gyax (1954) was followed, with the exception of step 4, which was omitted. The sections from one cat were stained with the Laidlaw modification of the Nauta technique (Chambers, Liu & Liu, 1956).

The original technique described by Nauta & Gyax (1951) followed the earlier method of Glees (1946) for the demonstration of degenerating axons. Both were important advances over the classical Marchi technique in that they made possible the study of terminal degeneration, but they suffered the disadvantage of staining many normal fibres as well. Subsequent papers of Nauta & Ryan (1952) and Nauta & Gyax (1954) and Chambers *et al.* (1956) described improvements, including a way of suppressing the staining of many or most of the normal fibres.

A lengthy discussion of the use of the Nauta technique and an interpretation of the results is made unnecessary here by the above-mentioned papers, and particularly by the recent review of Glees & Nauta (1955). Drop-like or varicose disintegration of the axons was made the essential criterion for the identification of both fibre and terminal degeneration. Proper use of the technique can render sections totally free of precipitate, although with experience, artifacts, when present, are easily distinguished from degeneration. In most cases the possibility of retrograde degeneration could be ruled out by the decreasing coarseness of the degenerating fibres, and their eventual termination on or near the cell bodies or dendrites. However, as in all degeneration techniques known to the authors, there are some fibres whose orientation cannot be determined with certainty.

The identification of individual, degenerating boutons cannot usually be made with the Nauta technique. Thus, the specific varicosity which is the remains of a previous bouton could not be identified, except in a few, very favourable positions (Pl. 1, figs. 3, 4). The position of the degenerating, fine terminal arborizations on or

very close to the cells (soma and proximal dendrites) makes clear that the technique is visualizing the axons up to, and in some cases including, their synaptic terminals (see Pl. 1).

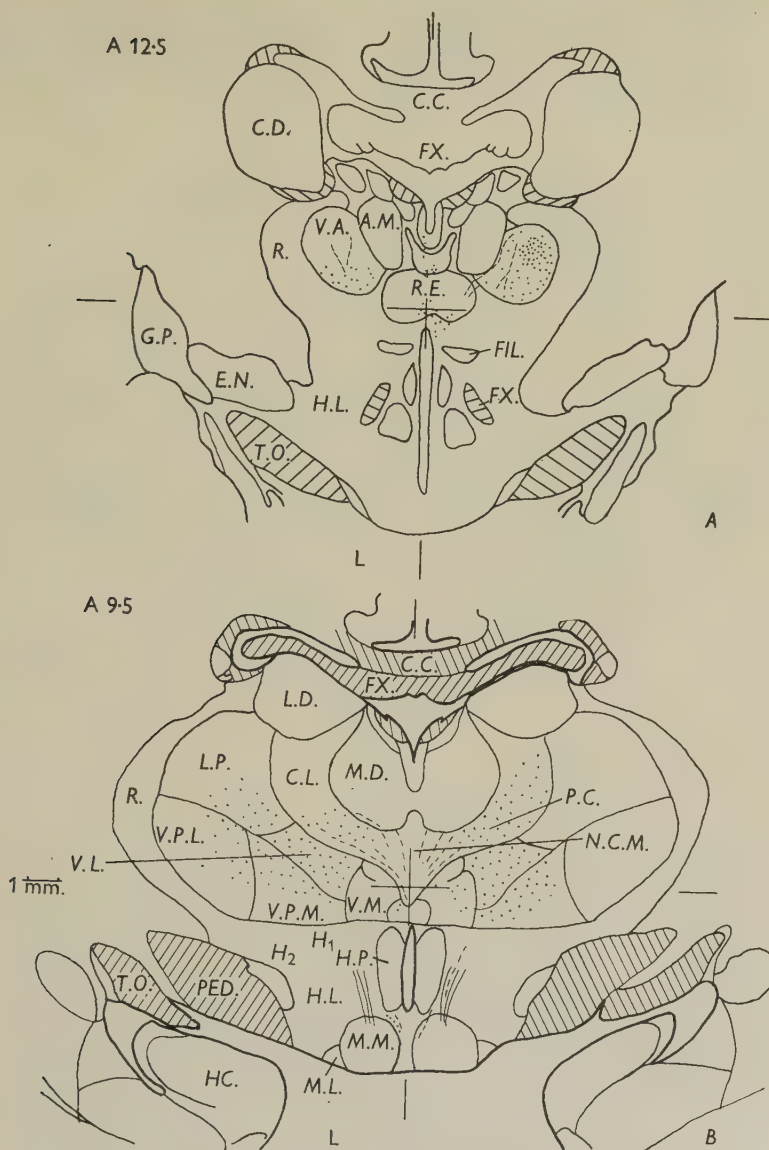
The distribution of degeneration from two animals is plotted on figures oriented for use in the stereotaxic instrument. Text-figs. 3 and 4(*E*) are reproduced with slight modifications from the atlas by Jasper & Ajmone-Marsan (1954). Those sections through the medulla are illustrated by Text-figs. 4(*F*), 5–7, taken from brain stems in the Northwestern University collection, and modified by the authors.

RESULTS

A subtotal fastigial lesion, which destroyed over three-fourths of the nucleus and spared only the ventral part and which was totally unilateral (Text-fig. 1) gave in two animals the pattern of degeneration shown in Text-figs. 2–7.

The terminology for the efferent projection of the fastigial nuclei used by Rand (1954) in the monkey will be followed here for the most part. Each nucleus contributes to the fastigiobulbar tract of both sides, associated with inferior and superior peduncles. The ipsilateral tract of each nucleus lies chiefly in the medial part of the inferior peduncle (juxta-restiform body), and has a small lateral portion which rostrally passes through and around the brachium conjunctivum (uncrossed uncinate fascicle). The crossed fastigiobulbar tract lies in its entirety in the lateral part of the restiform body, and rostrally passes through and around the brachium (crossed uncinate fascicle). Both uncinate fascicles, chiefly the crossed, give off fibres which pass out of the cerebellum in the dorsomedial part of the superior peduncles. These form the ascending projections of the fastigial nucleus and pass up the brain stem in that tract known as the uncrossed, ascending limb of the brachium (Rand, 1954; Carrea & Mettler, 1954). No part of the fastigiobulbar tract crosses outside of the cerebellum, except that on the posterior commissure.

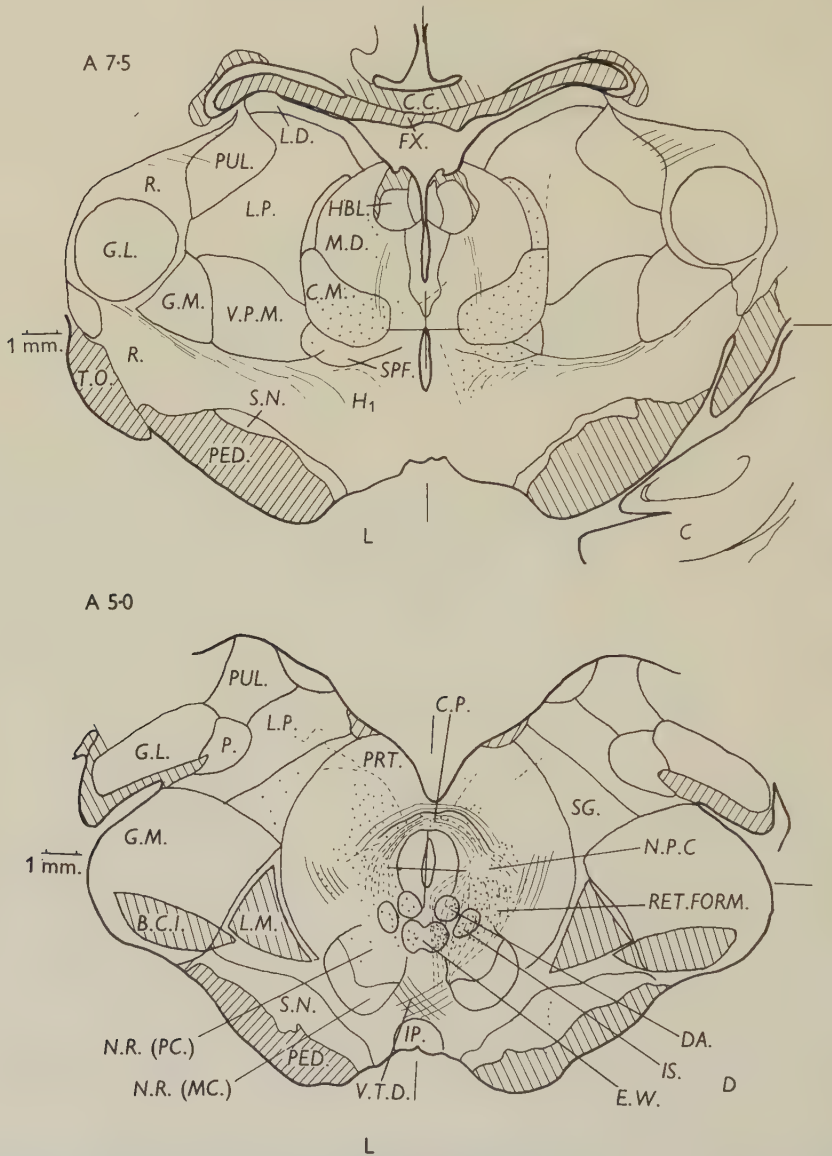
The course of these pathways and their terminations will be clear from the illustrations. A few points need emphasis. Different parts of the vestibular nuclei are involved on the two sides. The contralateral path largely avoids the superior vestibular nucleus and the dorsomedial part of the lateral vestibular nucleus. The ipsilateral fastigiobulbar tract terminates heavily on both of these areas, and largely avoids the ventrolateral portion of the lateral vestibular nucleus. Descending fibres lying chiefly in the dorsomedial part of Deiter's nucleus ipsilaterally and in the ventrolateral part contralaterally extend the fastigiobulbar projection caudally to the spinal vestibular nuclei and to the lower parts of the reticular formation in medulla and cervical cord (Text-figs. 6, 7). Terminal degeneration in the medullary reticular formation is chiefly medial and contralateral, and is found in both dorsal and ventral paramedian nuclei. Certain other nuclei which project to the cerebellum—lateral reticular, ventral arcuate, intercalatus and prepositus—receive small but unmistakable contributions from the fastigiobulbar tracts (Text-figs. 5*H*, 6, 7*K*). Sparse terminal degeneration found in the lateral reticular nucleus lies chiefly in that part which projects specifically to the vermal cortex. Also should be mentioned a small amount of degeneration in the nuclei on the lateral lemniscus, and in the dorsal cochlear nuclei (not shown in figures). The degenerating fibres lying in the



Text-fig. 2 (*A, B*) is from the thalamus, showing the distribution of the most rostral fibres from the fastigius. Fibre degeneration, shown by broken lines, terminal degeneration by dots. L marks the side of lesion.

Text-figs. 2-7. Drawings of sections cut transversely in planes of the stereotaxic instrument, the levels being indicated in millimetres anterior (A) and posterior (P) to interaural zero. The intersection of vertical and horizontal planes is shown, as is the scale of 1 mm. Drawings *A-E* are reproduced with slight modifications from Jasper & Ajmone-Marsan 1954 (courtesy of National Research Council of Canada).

solitary tracts do not enter the solitary nuclei; their destination is unknown but appears to be the adjacent reticular formation. No degeneration was present in the inferior olive, the cranial nerve nuclei (sensory or motor) and the medial longitudinal

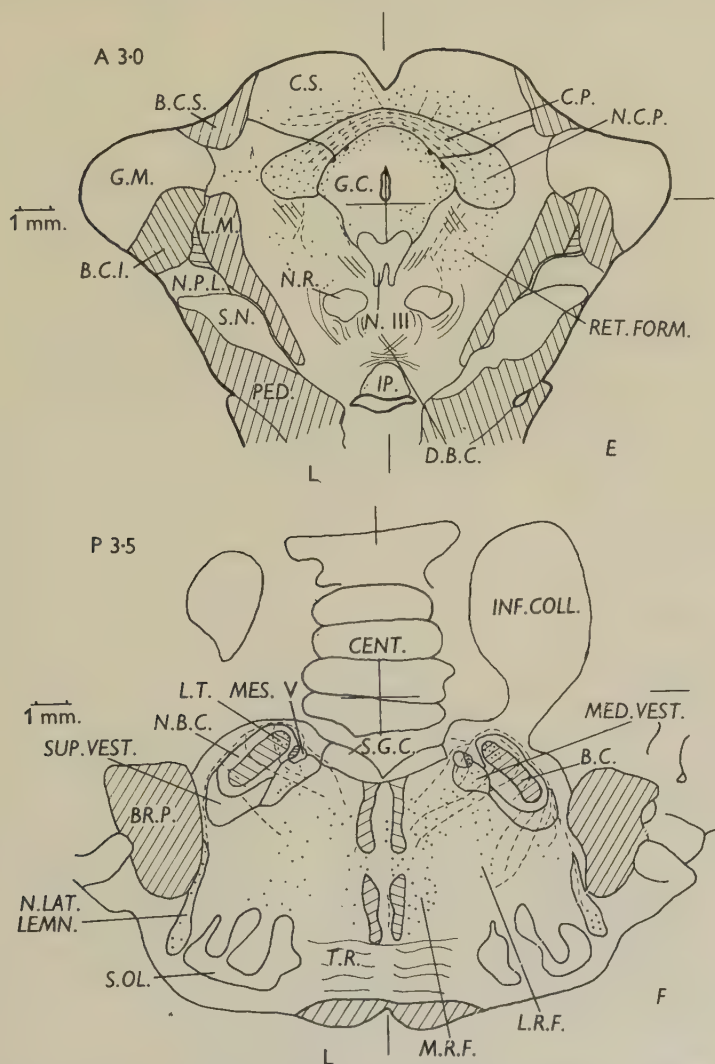


Text-fig. 3. Drawings of sections through the caudal thalamus (C), and rostral midbrain (D) showing the distribution of the fastigiobulbar tract.

fascicle. Likewise degeneration in the cervical spinal cord avoided the motor cells and was found only on interneurons in the intermediate and ventral grey matter. Degeneration from the fastigial nucleus to the cerebellar cortex was not mapped,

but degeneration could only be traced into the granular layer, and the basal part of the Purkinje layer. Thus climbing fibres could not be identified.

Certain fibres of the uncinate fascicles penetrate the brachia conjunctiva and leave the cerebellum in the dorsomedial part of this peduncle on both sides, chiefly on the

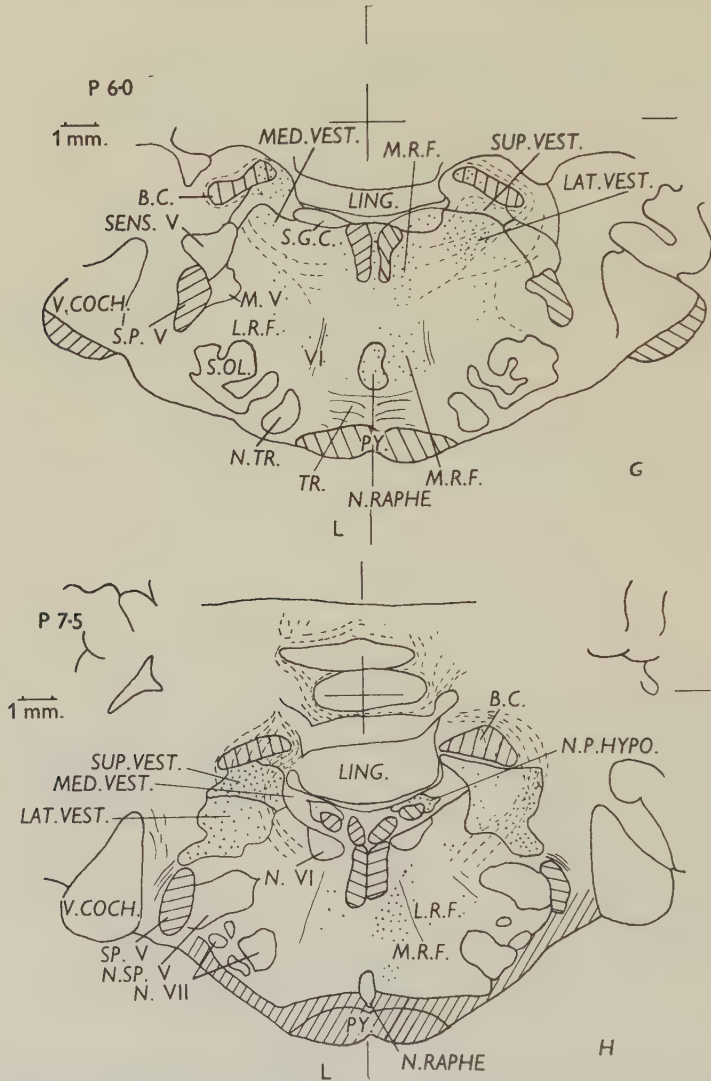


Text-fig. 4. Drawings of sections through the caudal midbrain (*E*) and the rostral medulla (*F*) showing the distribution of the fastigiobulbar tract.

contralateral (Text-figs. 4*F*, 5). These fastigial fibres ascend the brain stem in the reticular formation without passing into the brachial decussation. Their distribution is shown in Text-figs. 2, 3, 4*E*.

The virtual absence of degeneration from the red nucleus should be noted in view of the known projections to this structure from interposed and dentate nuclei. In

the thalamus, the fastigiobulbar terminations lie chiefly in the midline and intralaminar nuclei, but definite contributions are in addition found in three specific nuclei—those receiving the spinal and trigeminal lemnisci (ventral posterolateral and medial) and those receiving the dentate projection (ventral lateral).



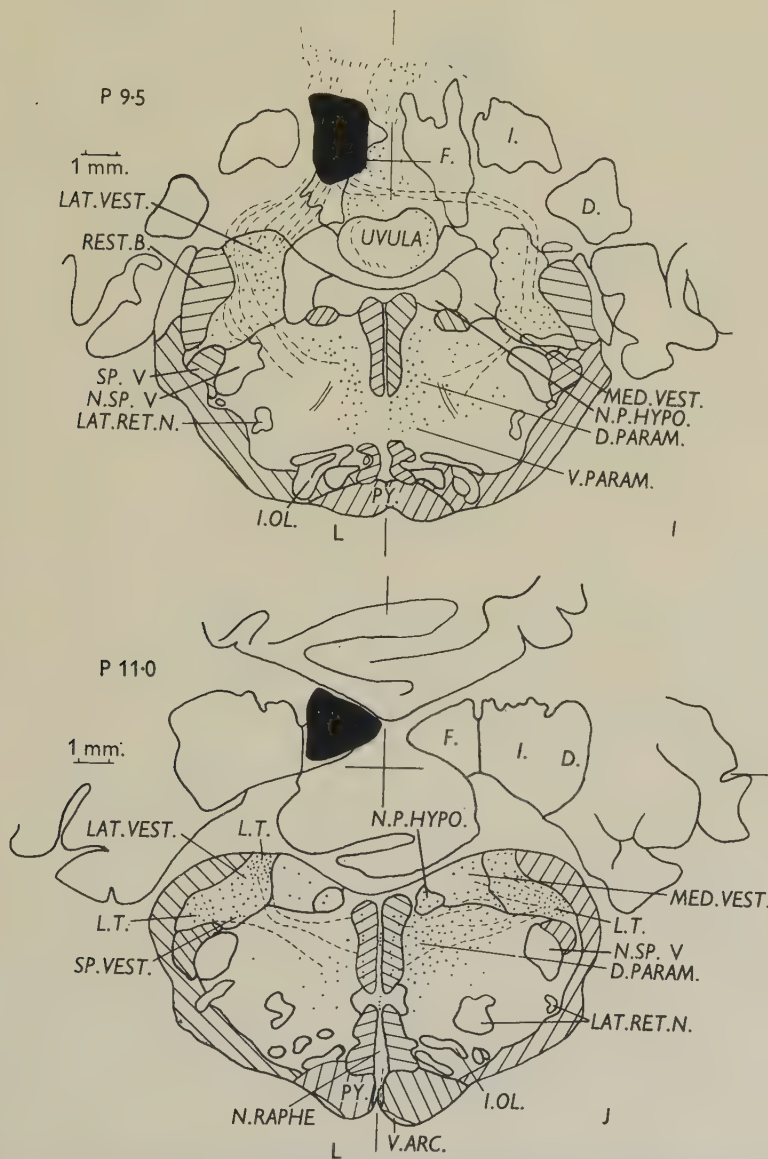
Text-fig. 5. Drawings of sections through the rostral half of the medulla (*G*, *H*) showing the distribution of the fastigiobulbar tract.

CONCLUSIONS

This study, based on a silver technique which selectively stains degenerating axons, has added many interesting details to the picture of the fastigial projection derived from use of the Marchi stain. The latter studies have been amply reviewed only recently by Jansen & Brodal (1954), Rand (1954), Carrea & Mettler (1954) and

Jansen & Jansen (1955), and the reader is referred to these excellent papers for a comprehensive coverage of the literature.

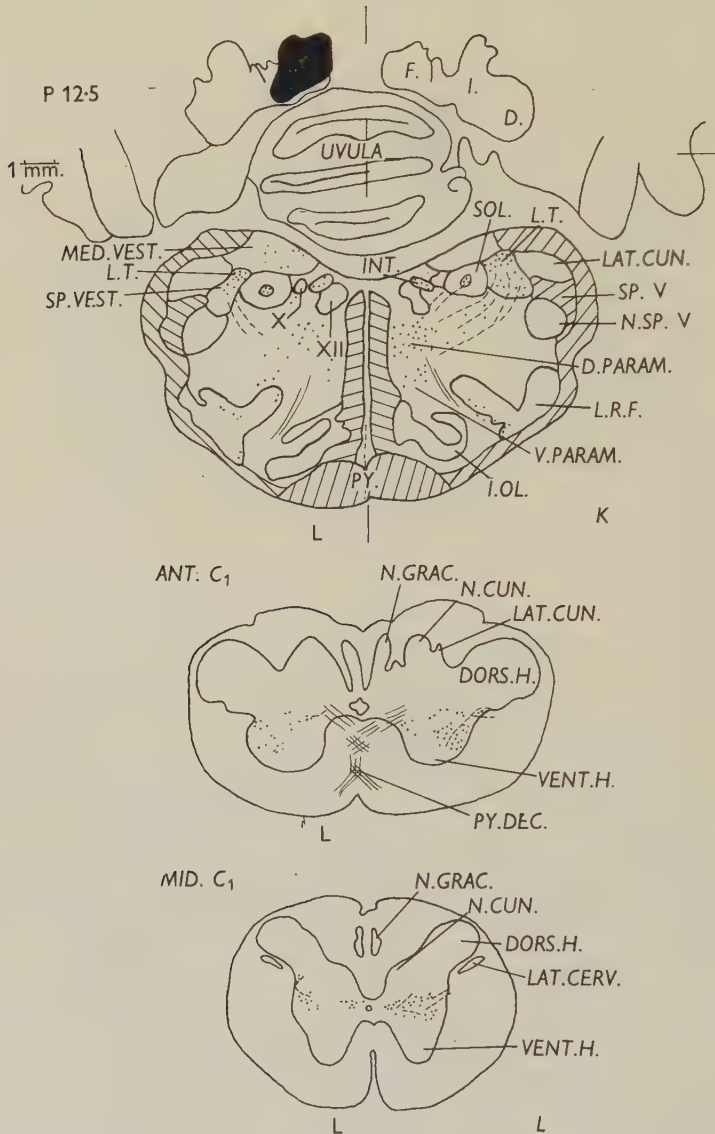
Because of the fact, emphasized by almost everyone who has worked in this area, that each fastigial nucleus has in addition to an ipsilateral projection a contralateral



Text-fig. 6. Drawings of sections through the caudal half of the medulla (I, J) showing the distribution of the fastigiobulbar tract.

path which passes *through* the opposite nucleus, no lesion can be considered as purely unilateral. The crossing fibres from the contralateral nucleus will be involved. Thus, this disadvantage of the present paper will be shared to a variable extent, dependent

on the size and position of the lesions, by most other workers discussed in the above papers. Jansen & Jansen (1955) have avoided this difficulty by studying retrograde degeneration in the nuclei after sectioning the efferent pathway at various levels.



Text-fig. 7. Drawings of sections through the caudal medulla (*K*) and the first cervical segment (*L*) showing the distribution of the fastigiobulbar tract.

Their study, which of course does not supply details of the types and areas of terminations, nevertheless supplements the picture obtained by nuclear lesions in a most useful way.

Our unilateral lesions, which spared the ventral part of the nucleus, clearly evoked more degeneration in the ipsilateral restiform body (direct tract) and in the contralateral uncinate fasciculus (indirect tract). How much the ipsilateral tract was supplemented by crossing fibres from the intact nucleus cannot easily be determined. Rasmussen (1933) believed that this decussation occurred chiefly in the dorsal part of the rostral poles of the nuclei, and our results confirm him in this respect. However, our lesions were placed with horizontally oriented electrodes (in contrast to his vertical lesions) which procedure minimized the involvement of the fibres decussating dorsal to the nucleus. The courses of crossed and uncrossed tracts agree with the description given by Rasmussen (1933) and need not be repeated here. The distribution of terminal degeneration is similar on the two sides of the medulla, but with certain differences in detail (Text-figs. 4F, 5, 6, 7K). The superior and lateral vestibular nuclei were more heavily involved ipsilaterally, and the medial and spinal vestibular nuclei contralaterally. Deiter's nucleus was divided into two parts by its fastigial projection, this being chiefly in the dorsal part of the nucleus on the same side and chiefly in the ventral part on the opposite side. That a subdivision of Deiter's nucleus is not limited to its cerebellar connexions is indicated by the work of Yoshida (1924), who found chromatolysis of the entire ipsilateral nucleus but of only the ventral part contralaterally following high cervical hemisection. The reticular formation of the medulla shows terminal degeneration from its rostral pole to the decussation of the pyramids, and is chiefly on the side opposite the fastigial lesion. The great predominance of the degeneration in the medial area on both sides is of particular interest in view of the similarity of postures evoked by stimulation of fastigial nuclei and medial reticular formation (Sprague & Chambers, 1954; Gernandt & Thulin, 1955). Most of the degenerating fibres which reached the lower medulla did so by means of descending paths lying in the substance of the spinal vestibular nuclei. Some degeneration, however, was consistently found in the position of the crossed descending limb of the brachium conjunctivum as defined by Rasmussen (1933), Carrea & Mettler (1954) and Rand (1954).

Our results differed from some authors in the lack of degeneration in any cranial motor nucleus. Of the medullary nuclei having cerebellar projections (see Jansen & Brodal, 1954) only the paramedian reticular nuclei whose primary cerebellar projection is the vermal zone received a significant contribution from the fastigiobulbar tract. Only that part of the lateral reticular nucleus which projects to the vermal cerebellum receives fibres from the fastigius and these are not numerous. Equally sparse is the degeneration in the intercalatus, prepositus and ventral arcuate nuclei, which also have their primary cerebellar projection to the vermal zone. No degeneration was found in the laterate cuneate nucleus and the inferior olive.

Evidence that the fastigial nucleus sends fibres out of the cerebellum via the superior peduncle has been on hand since Probst, 1902 (cited by Rand, 1954). Our confirmatory finding in the cat has been supported recently by Jansen & Jansen (1955), who believed this tract to originate entirely from the caudal one-third of the contralateral fastigius. Our material shows degeneration in and capping the dorso-medial tip of both brachia, especially the contralateral one. This efferent path of the fastigius does not enter the decussation of the brachium conjunctivum, but joins the so-called uncrossed ascending limb of the brachium, whose path in the lateral

reticular formation is well described by Rasmussen (1933), Carrea & Mettler (1954), Jansen & Brodal (1954) and Rand (1954). The location of this tract obviously is the same as that described by Russell (1954) as the lateral reticular thalamic tract, an ascending pathway originating in the reticular formation of the rostral medulla. Thus the fastigius does not send fibres to the red nucleus, nor does it receive fibres from this nucleus (Jansen & Brodal, 1954). Thus fibres in the ascending limb of the brachium originate not only in the cerebellum (including the fastigial nucleus) but also in the medullary reticular formation. Some of the terminations of these two components appear to be similar. The present results show (Text-figs. 2-7) an ascending field of degeneration which terminates in the mesencephalic reticular formation, nuclei of posterior commissure, posterior commissure, superior colliculus, nuclei of Edinger-Westfall and Darkschewitsch, interstitial and pretectal nuclei, Forel's field H_1 , and subparafascicular, centre median, central lateral, ventral anterior, ventrolateral, ventral posterolateral and medial and lateral posterior thalamic nuclei. This extensive degeneration appears disproportionate to the small number of fibres in and around the dorsomedial pole of the brachium. An ascending contribution from the medullary reticular formation via the lateral reticular thalamic tract (see above) is not unlikely.

SUMMARY

Degenerated fibres resulting from near total lesions of one fastigial nucleus in the cat were followed to their terminations by means of the Laidlaw modification of the Nauta silver technique.

Each fastigial nucleus has its outflow (fastigiobulbar tract) via the inferior and superior peduncles of both sides. The ipsilateral part of the tract is chiefly direct to all vestibular nuclei (less to reticular formation) by way of the juxta-restiform body. The contralateral part of the tract is chiefly indirect to the reticular formation (less to vestibular nuclei) by way of the uncinate fasciculus.

Both the crossed and the smaller uncrossed uncinate fascicles give fibres to the dorsomedial part of the brachia conjunctiva, which fibres pass through the mesencephalon on each side in the so-called uncrossed, ascending limb of the brachium. All decussation in the fastigiobulbar tract occurs in the cerebellum, with the exception of that in the posterior commissure.

The field of degeneration, far more extensive than previously reported, extends from the rostral thalamus throughout the brain stem into the upper cervical segments of the spinal cord. No degeneration is found in somatic motor nuclei at any point, nor in any primary sensory nuclei (except vestibular, and to a small extent cochlear). The major medullary reticular projection is medial.

The degeneration above the medulla is primarily contralateral, and terminates in the following nuclear masses: Edinger-Westfall, interstitial, Darkschewitsch, posterior commissure, tegmental reticular formation, pretectal, superior colliculus, Forel field H_1 , ventral posterolateral and medial, ventral lateral, subparafascicular, centralis lateral, centre median, reuniens and ventral anterior.

This investigation was supported (in part) by research grants (B-49, B-241) from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

The authors wish to thank Miss Elizabeth Hunter for invaluable technical assistance. We are indebted to Prof. Jansen and Prof. Brodal, Anatomical Institute, University of Oslo, for permission to reproduce Text-fig. 1, and to Drs Jasper and Ajmone-Marsan for permission to use drawings in Text-figs. 2, 3, 4E. The photomicrographs were taken by Mr Carlyle, Rockefeller Institute for Medical Research, New York City.

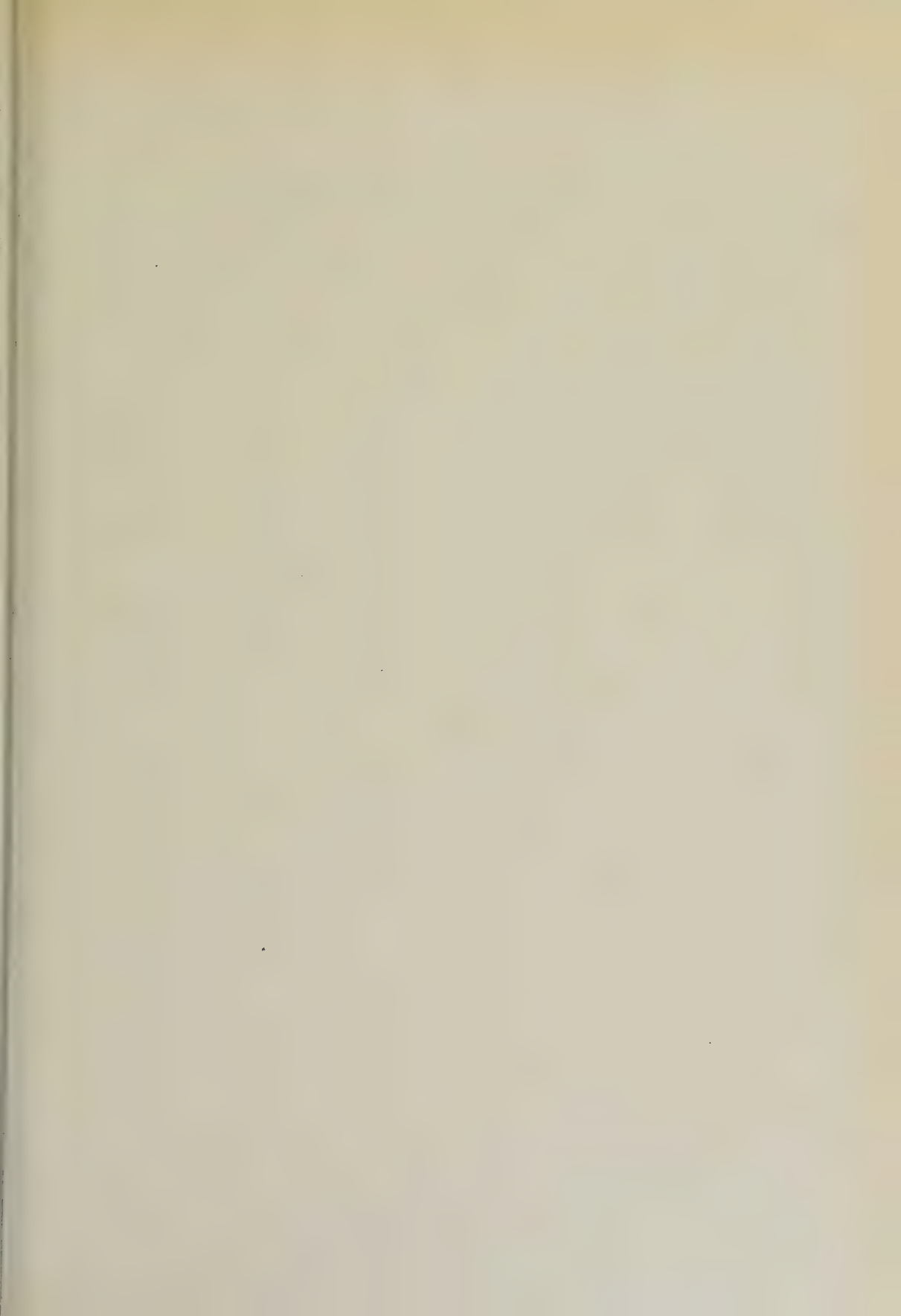
REFERENCES

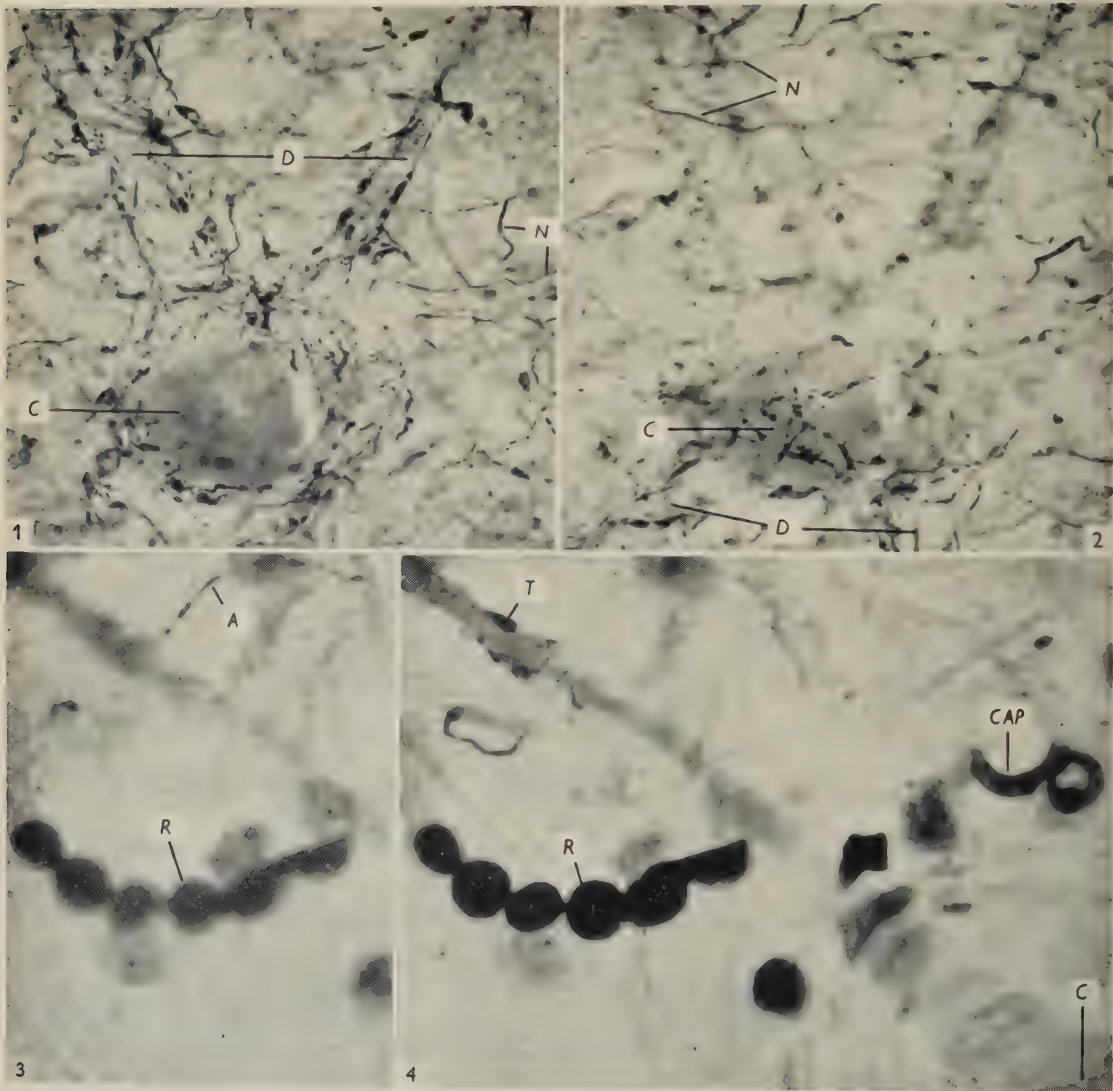
- ALLEN, W. F. (1924). Distribution of fibers originating from the different basal cerebellar nuclei. *J. comp. Neurol.* **36**, 399-439.
- CARREA, R. M. E. & METTLER, F. A. (1954). The anatomy of the primate brachium conjunctivum and associated structures. *J. comp. Neurol.* **101**, 565-689.
- CHAMBERS, W. W. (1947). Electrical stimulation of the interior of the cerebellum in the cat. *Amer. J. Anat.* **80**, 55-94.
- CHAMBERS, W. W., LIU, C. & LIU, J. (1956). A modification of the Nauta technique for staining of degenerating axons in the central nervous system. *Anat. Rec.* (In the Press.)
- CHAMBERS, W. W. & SPRAGUE, J. M. (1955a). Functional localization in the cerebellum: I. Organization in longitudinal cortico-nuclear zones and their contribution to the control of posture, both extrapyramidal and pyramidal. *J. comp. Neurol.* **103**, 105-129.
- CHAMBERS, W. W. & SPRAGUE, J. M. (1955b). Functional localization in the cerebellum: II. Somatotopic organization in cortex and nuclei. *Arch. Neurol. Psychiat. Chicago*, **74**, 653-680.
- COHEN, D., CHAMBERS, W. W. & SPRAGUE, J. M. (1956). The efferent projection of the interposed and dentate nuclei in the cat. (In preparation.)
- DOW, R. S. (1942). The evolution and anatomy of the cerebellum. *Biol. Rev.* **17**, 179-220.
- FULTON, J. F. & DOW, R. S. (1937). The cerebellum: a summary of functional localization. *Yale J. Biol. Med.* **10**, 89-119.
- GERNANDT, B. E. & THULIN, C. A. (1955). Reciprocal effects upon spinal motoneurons from stimulation of bulbar reticular formation. *J. Neurophysiol.* **18**, 113-129.
- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath.* **5**, 54-59.
- GLEES, P. & NAUTA, W. J. H. (1955). A critical review of studies on axonal and terminal degeneration. *Msschr. Psychiat. Neurol.* **129**, 74-91.
- INGVAR, S. (1923). On cerebellar localization. *Brain*, **46**, 301-335.
- JANSEN, J. & BRODAL, A. (1940). Experimental studies on the intrinsic fibers of the cerebellum. II. The cortico-nuclear projection. *J. comp. Neurol.* **73**, 267-321.
- JANSEN, J. & BRODAL, A. (1942). Experimental studies on the intrinsic fibers of the cerebellum. III. The cortico-nuclear projection in the rabbit and the monkey. *Avh. norske VidenskAkad.* **3**, 3-50.
- JANSEN, J. & BRODAL, A. (1954). *Aspects of Cerebellar Anatomy*. Oslo: Tanum.
- JANSEN, J. & JANSEN, J., JR. (1955). On the efferent fibers of the cerebellar nuclei in the cat. *J. comp. Neurol.* **102**, 607-632.
- JASPER, H. H. & AJMONE-MARSAN, C. (1954). *A Stereotaxic Atlas of the Diencephalon of the Cat*. Ottawa: National Research Council of Canada.
- KOENIG, H., GROAT, R. A. & WINDLE, W. F. (1945). A physiological approach to perfusion-fixation of tissues with formalin. *Stain Tech.* **20**, 13-22.
- LARSELL, O. (1937). The cerebellum: a review and interpretation. *Arch. Neurol. Psychiat. Chicago*, **38**, 580-607.
- MORUZZI, G. (1950). *Problems in Cerebellar Physiology*. Springfield: Thomas.
- MUSSEN, A. T. (1927). Experimental investigations on the cerebellum. *Brain*, **50**, 313-349.
- NAUTA, W. J. H. & GYGAX, P. A. (1951). Silver impregnation of degenerating axon terminals in the central nervous system. *Stain Tech.* **26**, 5-11.
- NAUTA, W. J. H. & GYGAX, P. A. (1954). Silver impregnation of degenerating axons in the central nervous system. *Stain Tech.* **29**, 91-93.

- NAUTA, W. J. H. & RYAN, L. F. (1952). Selective silver impregnation of degenerating axons in the central nervous system. *Stain Tech.* **27**, 175-179.
- RAMON Y CAJAL, S. (1909). *Histologie du système nerveux de l'homme et des vertébrés*. Vol. 1. Paris: Maloine.
- RAND, R. W. (1954). An anatomical and experimental study of the cerebellar nuclei and their efferent pathways in the monkey. *J. comp. Neurol.* **101**, 167-224.
- RASMUSSEN, A. T. (1933). Origin and course of the fasciculus uncinatus (Russell) in the cat, with observations on other fiber tracts arising from the cerebellar nuclei. *J. comp. Neurol.* **57**, 165-184.
- RUSSELL, G. V. (1954). The dorsal trigemino-thalamic tract in the cat reconsidered as a lateral reticulo-thalamic system of connections. *J. comp. Neurol.* **101**, 237-263.
- SPRAGUE, J. M. & CHAMBERS, W. W. (1953). Regulations of posture in intact and decerebrate cat. I. Cerebellum, reticular formation, vestibular nuclei. *J. Neurophysiol.* **16**, 451-463.
- SPRAGUE, J. M. & CHAMBERS, W. W. (1954). Control of posture by reticular formation and cerebellum in the intact, anesthetized and unanesthetized, and in the decerebrate cat. *Amer. J. Physiol.* **176**, 52-64.
- YOSHIDA, I. (1924). Ein Beitrag zur Kenntnis der zentralen Vestibularisbahn. *Folia anat. Japon.* **2**, 283-288.
- ZANCHETTI, A. & ZOCCOLINI, A. (1954). Autonomic hypothalamic outbursts elicited by cerebellar stimulation. *J. Neurophysiol.* **17**, 475-483.

KEY TO ABBREVIATIONS

<i>A.M.</i>	Anterior medial nucleus	<i>LAT.RET.N.</i>	Lateral reticular nucleus
<i>B.C.</i>	Brachium conjunctivum	<i>LAT.VEST.</i>	Lateral vestibular nucleus
<i>B.C.I.</i>	Brachium inferior colliculus	<i>L.D.</i>	Lateral dorsal nucleus
<i>B.C.S.</i>	Brachium superior colliculus	<i>LING.</i>	Lingula, anterior lobe
<i>BR.P.</i>	Brachium pontis	<i>LM.</i>	Medial lemniscus
<i>C.C.</i>	Corpus callosum	<i>L.P.</i>	Lateral posterior nucleus
<i>C.D.</i>	Caudate nucleus	<i>L.R.F.</i>	Lateral reticular formation
<i>CENT.</i>	Centralis, anterior lobe	<i>L.T.</i>	Indicates degeneration of longitudinal tract
<i>C.L.</i>	Central lateral	<i>M.V.</i>	Motor trigeminal nucleus
<i>C.M.</i>	Centromedian	<i>M.D.</i>	Medial dorsal nucleus
<i>C.P.</i>	Posterior commissure	<i>MED.VEST.</i>	Medial vestibular nucleus
<i>C.S.</i>	Superior colliculus	<i>MES.V</i>	Mesencephalic trigeminal nucleus
<i>D.</i>	Dentate nucleus	<i>M.L.</i>	Lateral mammillary nucleus
<i>DA.</i>	Darkschewitsch nucleus	<i>M.M.</i>	Medial mammillary nucleus
<i>D.B.C.</i>	Decussation brachium conjunctivum	<i>M.R.F.</i>	Medial reticular formation
<i>DORS.H.</i>	Dorsal horn	<i>N. III</i>	Oculomotor nucleus
<i>D.PARAM.</i>	Dorsal paramedian reticular nucleus	<i>N. VI</i>	Abducens nucleus
<i>E.N.</i>	Entopeduncular nucleus	<i>N. VII</i>	Facial nucleus
<i>E.W.</i>	Edinger-Westfall nucleus	<i>N.B.C.</i>	Nucleus brachium conjunctivum
<i>F.</i>	Fastigial nucleus	<i>N.C.M.</i>	Central medial
<i>FIL.</i>	Filiform nucleus	<i>N.C.P. (N.P.C.)</i>	Posterior commissure nucleus
<i>FX.</i>	Fornix	<i>N.CUN.</i>	Cuneate nucleus
<i>G.C.</i>	Central grey substance mid-brain	<i>N.GRAC.</i>	Gracile nucleus
<i>G.L.</i>	Lateral geniculate nucleus	<i>N.LAT.LEMN.</i>	Lateral lemniscus nucleus
<i>G.M.</i>	Medial geniculate nucleus	<i>N.P.HYPO.</i>	Prepositus nucleus
<i>G.P.</i>	Globus pallidus	<i>N.P.L.</i>	Paralemniscal nucleus
<i>H₁ and H₂</i>	Forel's field	<i>N.R.</i>	Red nucleus
<i>HBL.</i>	Habenula	<i>N.R. (MC.)</i>	Magnocellular part
<i>HC.</i>	Hippocampus	<i>N.R. (PC.)</i>	Parvocellular part
<i>H.L.</i>	Lateral hypothalamus	<i>N.RAPHE.</i>	Raphe nucleus
<i>H.P.</i>	Posterior hypothalamus	<i>N.SP. V</i>	Spinal trigeminal nucleus
<i>I.</i>	Interposed nucleus	<i>N.TR.</i>	Trapezoid nucleus
<i>INF.COLL.</i>	Inferior colliculus	<i>P.</i>	Posterior nucleus
<i>INT.</i>	Intercalated nucleus	<i>P.C.</i>	Paracentral nucleus
<i>I.OL.</i>	Inferior olivary nucleus	<i>PED.</i>	Cerebral peduncle
<i>I.P.</i>	Interpeduncular nucleus	<i>PRT.</i>	Pretectal nucleus
<i>IS.</i>	Interstitial nucleus	<i>PUL.</i>	Pulvinar
<i>LAT.CERV.</i>	Lateral cervical nucleus	<i>PY.</i>	Pyramid
<i>LAT.CUN.</i>	Lateral cuneate		





THOMAS AND OTHERS—VERMAL CEREBELLAR PROJECTIONS IN BRAIN STEM OF THE CAT

(Facing p. 385)

<i>PY.DEC.</i>	Decussation, pyramid	<i>T.O.</i>	Optic tract
<i>R.</i>	Reticular nucleus, thalamus	<i>TR.</i>	Trapezoid body
<i>R.E.</i>	Reuniens nucleus	<i>V.A.</i>	Ventral anterior nucleus
<i>REST.B.</i>	Restiform body	<i>V.ARC.</i>	Ventral arcuate nucleus
<i>RET.FORM.</i>	Reticular formation	<i>V.COCH.</i>	Ventral cochlear nucleus
<i>SENS. V</i>	Sensory trigeminal nucleus	<i>VENT.H.</i>	Ventral horn
<i>SG.</i>	Supragenulate nucleus	<i>VI.</i>	Abducens nerve
<i>S.G.C.</i>	Central grey substance, medulla	<i>V.L.</i>	Ventral lateral nucleus
<i>S.N.</i>	Substantia nigra	<i>V.M.</i>	Ventral medial nucleus
<i>SOL.</i>	Solitary nucleus and tract	<i>V.PARAM.</i>	Ventral paramedian reticular nucleus
<i>S.OL.</i>	Superior olivary nucleus	<i>V.P.L.</i>	Ventral posterior lateral nucleus
<i>SP. V</i>	Spinal trigeminal nucleus and tract	<i>V.P.M.</i>	Ventral posterior medial nucleus
<i>SPF.</i>	Subparafascicular nucleus	<i>V.T.D.</i>	Ventral tegmental decussation
<i>SP.VEST.</i>	Spinal vestibular nucleus	<i>X and XII</i>	Vagal and hypoglossal nuclei
<i>SUP.VEST.</i>	Superior vestibular nucleus		

EXPLANATION OF PLATE

Figs. 1 & 2. Photomicrographs ($\times 660$) of a multipolar cell of Deiter's nucleus, taken at two focal planes to show the terminal degeneration on dendrites and cell body (compare with Ramon y Cajal, 1909, fig. 321). *N*=normal fibres, *C*=cell body, *D*=basal dendrites.

Figs. 3 & 4. Photomicrographs ($\times 2209$) of a dendrite of a large cell in the fifth layer of the superior colliculus, taken in two focal planes to show a single degenerating axon (*A*), and its cluster of degenerating terminals (*T*). *CAP*=capillary, *R*=erythrocytes, *C*=cell body. In Fig. 4 the continuation of the fibre labelled *A* in Fig. 3 can be seen joining the cluster of degenerating terminals just below and to the right of the label *T*.

A STUDY OF THE DEVELOPMENT OF THE CEREBRAL CORTEX OF THE FOETAL GUINEA-PIG BY MEANS OF THE ULTRA-VIOLET MICROSCOPE

BY ARTHUR HUGHES AND LOUIS B. FLEXNER

*The Anatomy School, Cambridge, and the Department of Anatomy,
School of Medicine, University of Pennsylvania*

One of the activities in embryology during recent years has been the study of how the differentiation of a cell leads to the assumption of a functional state which foreshadows its behaviour and purpose in the fully developed organism. Among examples of investigations of this kind are researches on the endocrine organs, recently reviewed by Willier (1955), and on the nervous system, where studies on the frontal cortex of the foetal guinea-pig have revealed information on the course of maturation of the cortical neurones which have been obtained by a variety of approaches (Flexner, 1952). By histological methods, observations have been made of the growth in size of the neurones, their affinity for silver, and the time at which the Nissl substance is first deposited. Parallel investigations by biochemical techniques have shown the rate at which some of the main enzyme systems within the nerve cells gradually assume their full scale of operation. Other researches have disclosed the time at which the characteristic electrical properties of the cortical neurones are first perceptible (Flexner, Tyler & Gallant, 1950).

From all of these investigations, the conclusion has emerged that many of these features of cortical development in the guinea-pig are first apparent at a point about two-thirds of the way through gestation. Soon after 40 days post coitus the nuclei of the neurones attain their final volume, Nissl substance first appears in the cytoplasm of the perikarya, the activity of such enzymes as succinic dehydrogenase, cytochrome oxidase and apyrase begins to increase; shortly afterwards the electrical activity of the cortex is first detectable. Among other biochemical studies on the foetal cortex, Flexner & Flexner (1951) have measured its concentration of nucleic acids, both DNA and RNA. With certain assumptions, measurements of concentration of RNA were combined with measurements of amount of cytoplasm of nerve cell bodies to give an estimate throughout the development of the cortex of the average amount of RNA per unit weight of cytoplasm of nerve cell bodies. At the 30th day of gestation this value is relatively high, but towards the 40th day it begins to decrease, and reaches a minimum at 55 days. Subsequently, values rise once again as term approaches. This means that the stages of maturation of the neurones which follow the 40th day occur in a period of falling average concentration of RNA in their cytoplasm. The first Nissl bodies are deposited at a time when the average density of cytoplasmic nucleic acid is decreasing.

Some recent work at Cambridge on the developing spinal cord of the chick (Hughes, 1955, 1956) suggests a parallel in the general course of development between the neurones of the mammalian cortex and of the avian cord.

This study on the nervous system of the chick has so far been confined to the exercise of a single technique, that of ultra-violet photomicrography. At wave-lengths near 2600 Å., absorption in animal cells may, with due precautions, be assigned wholly to ribonucleic acid. In this way, distribution of this substance may be studied free of the variables introduced by staining methods.

The use of the ultra-violet microscope for the study of the developing neurone was begun by Hydén (1943). His paper belongs to that early group of studies by Caspersson and his school from which our current ideas on the function and purpose of the nucleic acids within the cell are largely derived (Caspersson, 1950). Hydén showed that, as in other young embryonic cells, the cytoplasmic RNA of neuroblasts in the cord of the rat foetus is concentrated around the nuclear membrane, and in them takes the form of a cap of densely absorbing material over one pole of the nucleus. In the neural tube of the chick embryo, this appearance is also seen in early stages of the differentiation of neuroblasts, at a time when they become monopolar in form; each has then a single primitive nerve fibre which has acquired an affinity for silver.

As this fibre grows in length, cytoplasmic RNA moves outwards from the perikaryon, where absorption then gradually becomes less intense. This change thus makes it possible to follow the differentiation of successive waves of neuroblasts within the cord. Both in cell-bodies and fibres absorption generally remains high until the end of the fifth day of incubation. At that time, every fibre in the white matter can clearly be discerned in an ultra-violet photomicrograph at the appropriate wave-length. During the sixth day, there is a sudden drop in contrast throughout the cord. At seven days, the blood cells within intra-neural capillaries absorb more strongly than do the surrounding nervous elements. In the succeeding period of development, however, contrast gradually increases once more within the neurones as the Nissl substance is deposited, though in a limited distribution restricted to the periphery of the perikaryon. This material is first detectable by staining with basic dyes on the ninth day.

These changes in the density of RNA in the neuro-cytoplasm of the chick cord were assessed semi-quantitatively by measurements of the absorption of the appropriate areas of the negatives obtained by ultra-violet photomicrography. The resulting points, when plotted against age of incubation, show a distribution closely similar to those obtained by Novikoff & Potter (1948) for the concentration of RNA in the whole embryo per unit of wet weight. For the embryo generally, the density of RNA falls to a sudden minimum at $6\frac{1}{2}$ days. This is later succeeded by a slow recovery.

These facts suggest a number of questions, of which two seem of primary importance. Are these changes within the neurones of the chick cord solely a reflexion of variations in concentration of RNA in the embryo as a whole during development, or have they also any wider significance? Are there elsewhere similar changes in the differentiation of neurones in other embryos?

The fact that both in the cerebral cortex of the foetal guinea-pig and in the neural tube of the chick embryo, the density of RNA in the neuro-cytoplasm passes through a minimum during development suggested that a closer comparison should be made of the differentiation of the neurones in both, in order to see what basis can be

assigned to this resemblance. It was thought that the first step in such an enquiry should be a study of the developing cerebral cortex by means of the ultra-violet microscope, so that data obtained by one method on both types of neurone could be compared. The purpose of this paper is to describe the results of this investigation.

MATERIAL AND METHODS

The guinea-pig foetuses were obtained from the colony which is maintained at Philadelphia by Dr L. B. Flexner. The same region of the cortex (areas *f'* and *f* of Fortuyn, 1914) was taken, on which the previous studies of the development of this material have been based. The technique of preparing the tissue was the same as that used in earlier work, except that the blocks of cortex were fixed and embedded by the same procedures which were used by Hughes (1955, 1956) in studying the cord of the chick embryo by means of the ultra-violet microscope, namely fixation in Serra's alcohol-formalin acetic mixture (Brachet, 1953), rapid dehydration in alcohol and 'cellosolve', and embedding in ester wax. The advantages of this procedure for this work have been discussed in the previous paper; an additional point for retaining it in the present instance was to facilitate the comparison of the observations obtained on the guinea-pig cortex and on the chick neural tube.

The material consisted of eight blocks, taken from six stages of development. These were as follows, reckoned in days p.c. 28, 33 (two specimens), 35 (two specimens), 40, 45 and one from a newborn animal. The blocks were sent to Cambridge, and were there sectioned at a standard thickness of 2.7μ . The sections were mounted on quartz slides, covered with a drop of liquid paraffin and a quartz coverslip, and photographed on 35 mm. film by means of a Cooke, Troughton and Simms ultra-violet microscope in the way described in Hughes (1956), after preliminary searching by means of an interchangeable phase-contrast optical system. Two objectives were available, a $\times 10$ quartz monochromat, and a $\times 81$ glycerin-immersion objective of the same type. With these lenses, the magnification of the negatives was respectively $\times 42$ and $\times 340$. At their best, they are capable of enlargement to a further ten diameters.

Recently the source of ultra-violet light has been improved by the acquisition of a more powerful mercury resonance lamp, thanks to the great kindness of Dr A. J. Vickers of the Thermal Syndicate Ltd. The radiation from this lamp is so narrowly restricted to a wave-length of 2537 Å. that a simple filter of the 'Wood's glass' type is sufficient to remove the bluish radiation which represents only a small part of the total emission. This visual light, however, has been found sufficient for the preliminary observation by phase-contrast, even when screened by a filter of green glass.

Even at the lower magnification, the flat central area of the field is no more than about 300μ in diameter, and so at all but the earliest stage it was found necessary to take a number of exposures along a line at right angles to the surface, in order to cover the full depth of the cerebral cortex. For the older material, up to eight such photographs were necessary, for each of which again a series of exposures at different focal depths were required, in order to ensure that the exact focus should be included. The total number of exposures necessary to cover the full depth of one cortex was often about fifty. From these negatives, the most suitable were printed,

and then assembled to give a complete section through the cortex. Occasional areas in these composite photographs where the focus is not exact indicate points where the coverage of the section was imperfect.

OBSERVATIONS

The composite photomicrographs of each stage of development of the cortex were compared with respect to the general changes in contrast which they show throughout the series. These are sufficiently well-marked to be adequately appreciated by qualitative comparison. They were closely collated with the description given by Peters & Flexner (1950) to whose account of the development of the cortex the observations described below are supplementary. The use by these authors of the Arabic numerals (1) and (3-6) for the layers of the foetal cortex has been adopted. In the adult guinea-pig, the second of Brodman's six cortical layers is absent (Fortuyn, 1914). Following Peters & Flexner (1950) the homology between the layers of the foetal and adult cortex implied by the use of the corresponding numerals is only provisional. In the descriptions which follow, the stages are referred to by their age in days p.c.

Twenty-eight days. The general contrast in the ultra-violet at this stage is relatively low (Pl. 1, fig. 1; Pl. 2, fig. 7). The cortex is about eight cells deep. These are densely packed, and as yet show no signs of stratification; their nuclei are all near a uniform size. At a high magnification, the nuclei are seen to be densely granular, with prominent nucleoli. There is conspicuous absorption around the nuclear membrane; this is often specially marked at one pole of the nucleus, usually that which faces inwards, where the cytoplasm is prolonged into a 'spiny expansion' (Peters & Flexner, 1950, p. 141), thus forming the cap of densely absorbing material characteristic of neuroblasts in their early stage of differentiation (Hydén, 1943; Hughes, 1955). Occasional fibres, radially orientated, can be seen between the cortical cells, but here, as in the adjacent layers, they are still sparsely distributed. Their contrast at 2537 Å. is sufficient to render them individually distinct. Both at this and at subsequent stages, some of the fibres visible in the ultra-violet photographs may be neuroglial.

No mitotic figures were seen either at this stage or at any subsequent period either in the cortex, or in the mantle layer beneath.

Thirty-three days. General contrast at this stage is still relatively low (Pl. 1, fig. 2; Pl. 2, fig. 8). The cortex has increased in depth about tenfold since the previous stage, and is now about 0.7 mm. thick. This increase is mainly due to the migration of cells into the cortex from the mantle layer, but also to the fact that the cells are now much less densely packed. As described by Peters & Flexner (1950) the cortex has now differentiated into three layers, nos. (3), (4) and (5). Of these (3) consists of cells rather similar to those of the whole cortex at earlier stages. The cells of layer (4) have increased markedly in size; their nuclei are round, and less dense than before, and are surrounded by a zone of 'grey' cytoplasm of moderate absorption. In layer (5), the nuclei are smaller and denser, but are also rounded. Nerve fibres are now seen throughout the cortex, and are prominent in layers (3) and (4), where they are uniformly radial in orientation. At high power, the finest

fibres are all clearly distinguishable. The irregular network of the molecular layer is now considerably denser.

Thirty-five days. The cortex is now about 0.9 mm. thick (Pl. 1, fig. 3; Pl. 2, fig. 9). The contrast at 2537 Å. has markedly increased throughout its depth during the last 2 days, and now approaches its maximum value. This is true equally of cell nuclei and cytoplasmic processes. In both, the difference between the stages of 33 and 35 days has been constant in all the sections which have been studied. Even in photomicrographs at low power, the fine fibres of the neuropil are recognizable, and the large ascending processes of layers (3) and (4) are particularly conspicuous. Their individual course can be traced within the depth of both layers. These processes often show a longitudinal striation which can be traced centrally into the perikaryon. Radial processes of the neurones of deeper layers are also clear, but they run for shorter distances than do those at more superficial levels. The contrast between the small ovoid, dense nuclei immediately beneath the molecular layer, and the larger rounded nuclei with a more open texture in layer (4) is still more marked than at earlier stages.

Forty days. The cortex is now about 1.0 mm. thick (Pl. 1, fig. 4). At low magnifications it is still possible to distinguish fine nerve fibres throughout, and the ascending processes of layers (3) and (4) are still generally evident, though in the largest neurones of layer (4) it is towards the perikaryon itself that their cytoplasm is most conspicuous. The nuclei of these cells are now still further enlarged. In layers (5) and (6), however, radial nerve processes are now more distinct than at 35 days. At this level the cells are now approximately at the stage which was reached two days earlier by those at more superficial levels. In the molecular layer, there have appeared numerous capillaries running at right angles to the surface of the cortex.

Forty-five days. The cortex is now about 1.15 mm. thick (Pl. 1, fig. 5; Pl. 2, fig. 10). The general contrast at 2573 Å. has by now begun to decrease, though some of the perikarya are now even more distinct. Although the fine fibres of the neuropil are still recognizable, especially in the upper layers of the cortex, yet much less is now seen of the proximal branches of the neurones. In the ultra-violet, only short lengths leading into the perikarya are now seen, despite the fact that, to quote Peters & Flexner (1950) 'the striking change from the preceding stage of development is the greater elaboration of processes by the nerve cells'. It is as though much of the RNA in the cytoplasm of the neurones was in retreat towards the perikaryon, where its density in some places is now greater than ever before, as the first traces of Nissl substance are there laid down.

Newborn; about 65 days p.c. The cortex is now about 1.45 mm. thick (Pl. 1, fig. 6; Pl. 2, fig. 11). The changes which were noticed at the preceding stage have continued, with the result that by now, about 20 days later, individual nerve fibres are no longer distinguishable by their absorption at 2537 Å., even under high magnification. The RNA is now so largely confined to the perikarya that where proximal nerve processes can be distinguished, it is because their contrast is even less than that of their surroundings. Only within the perikarya themselves is there a marked absorption; the irregular dense areas of the young Nissl bodies are distinguishable. There is now much less range in the size and shape of the nuclei. Their contents are still granular, and the nucleoli have remained very conspicuous throughout.

DISCUSSION

In comparing these observations on the developing cortex with the results described by Peters & Flexner (1950), one asks what extra information beyond that disclosed by standard histological methods has the use of the ultra-violet microscope revealed? The answer mainly concerns the first period of development, at which these authors (p. 134) state that for fine nerve fibres 'neither protargol nor thionin showed them adequately at early stages'. In the ultra-violet, however, they are then clearly visible. Although the dense cone of RNA over the nuclear membrane of the early neuroblast can be revealed by basic dyes, yet the more sparsely distributed material of the fibres and nerve processes then escapes notice in such preparations. Peters & Flexner (1950) found that the 'dust-like basophilia' of the cells of the cortex was not detectable before the 34th day; yet at 2537 A. their absorption is then approaching its maximum. This extension of the means of studying the early development of the neurone is perhaps sufficient to justify the use of the ultra-violet microscope in neuro-embryology.

In the cortex of the newborn guinea-pig, however, comparison of the results of Peters & Flexner (1950) with those obtained in the present study suggests that the definitive Nissl bodies of more mature stages are better revealed by the use of basic dyes. This difference in the effectiveness of the two methods in early and late stages of foetal life may be due to changes in composition and molecular arrangement of the RNA during the course of development, possibly those which finally lead to the appearance of the metachromatic reaction of the Nissl body.

The curve given in Flexner & Flexner (1951) for the ribonucleic acid phosphorus per unit weight of perikaryon in the developing cortex shows features which correspond with the results obtained during the course of the present study. For instance, the points for 35 and 40 days are well above those for 45 days and at term; the general changes in contrast within the ultra-violet photographs agree with those which their values for these stages would suggest. These authors, for the sake of their calculations, made the assumption that 'all the cytoplasmic RNA belongs to the perikaryon'. Although this must be revised in the light of the present findings, there is no ready means of quantitative measurement of the distribution of RNA between cells and fibres, and of the changes therein which occur during development.

We now come to a comparison of the general course of development of neurones in the cortex of the guinea-pig and in the spinal cord of the chick embryo. In both, there are two periods when RNA is conspicuous within the neurocytoplasm; first, an early phase of generalized absorption, which is at its maximum in the guinea-pig cortex at about 35 days p.c., and in the chick cord in the 5th day of incubation. This phase is separated by a period of minimum absorption from the later era when the Nissl bodies make their appearance. In the chick spinal cord and dorsal root ganglia, the end of the first period is sharply demarcated by the sudden general fall in RNA concentration during the seventh day; Nissl substance does not appear until 2-3 days later. In the cortex of the guinea-pig, an animal with a much more extended time-scale of development, the first phase of absorption is only slowly brought to an end, and overlaps with the first formation of the Nissl substance at a period about two-thirds of the way through gestation. Thanks to the gradual ending of this

first period, it can be seen that in these cortical neurones the later concentration of RNA in the perikarya is accompanied by a slow disappearance of this material from the proximal cell processes.

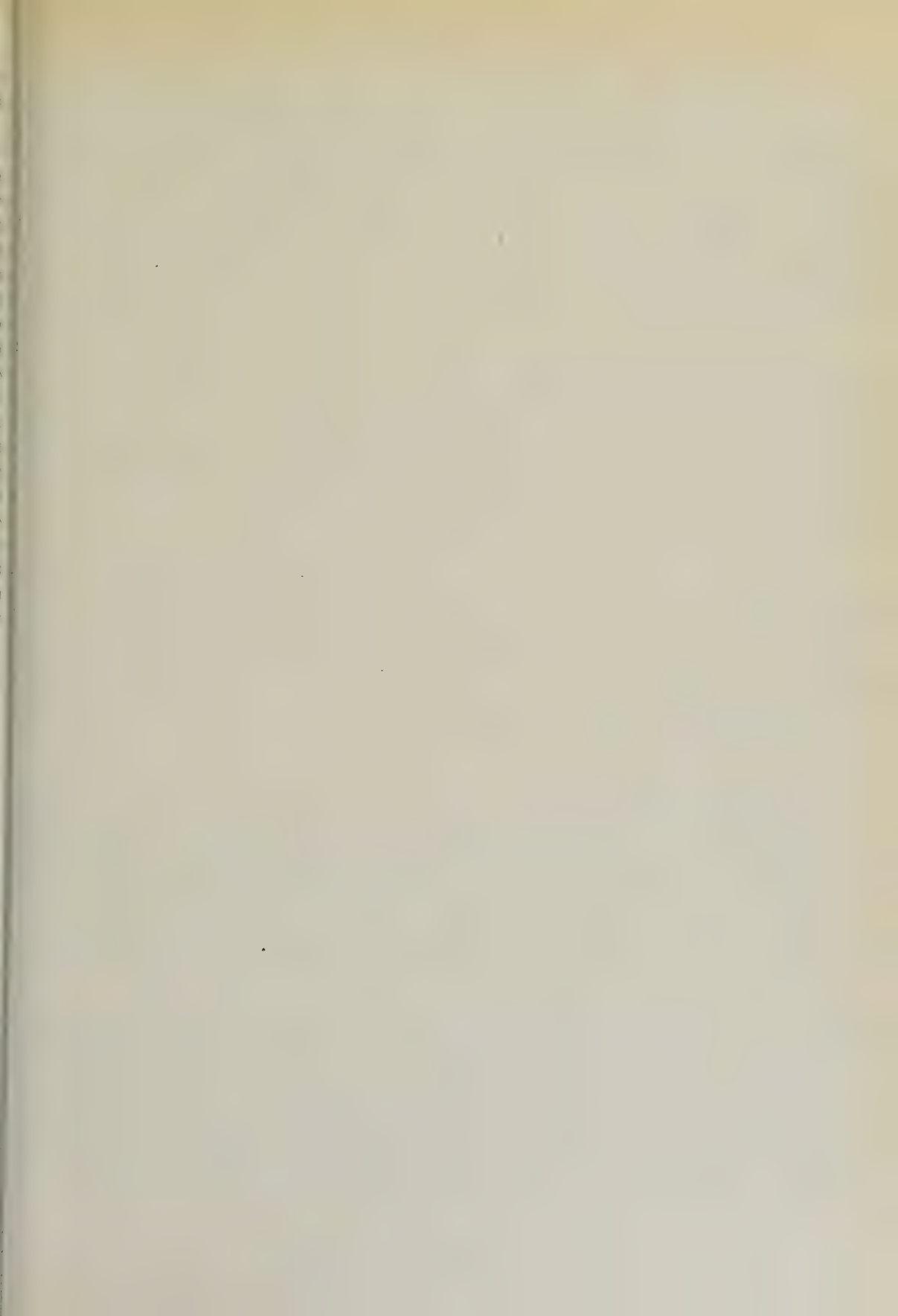
If we accept the general views of the Stockholm school and others that RNA is associated with protein syntheses, then the change in distribution of RNA between perikarya and dendrites may indicate a change in synthetic activity of those parts of the nerve cell. Thus in the earlier stages of development of nerve processes, we should infer that the dendrites are then participating importantly in protein synthesis and so adding to their own protein content. At later stages, when the perikarya contain the greater part of the RNA, synthesis has declined in the dendrites and is carried on in major part by the perikarya. The observation of Peters & Flexner (1950) on the increase of refractive index of the proximal processes of the cortical neurones from the 41st day are in all probability related to increase in protein concentration within the neurocytoplasm.

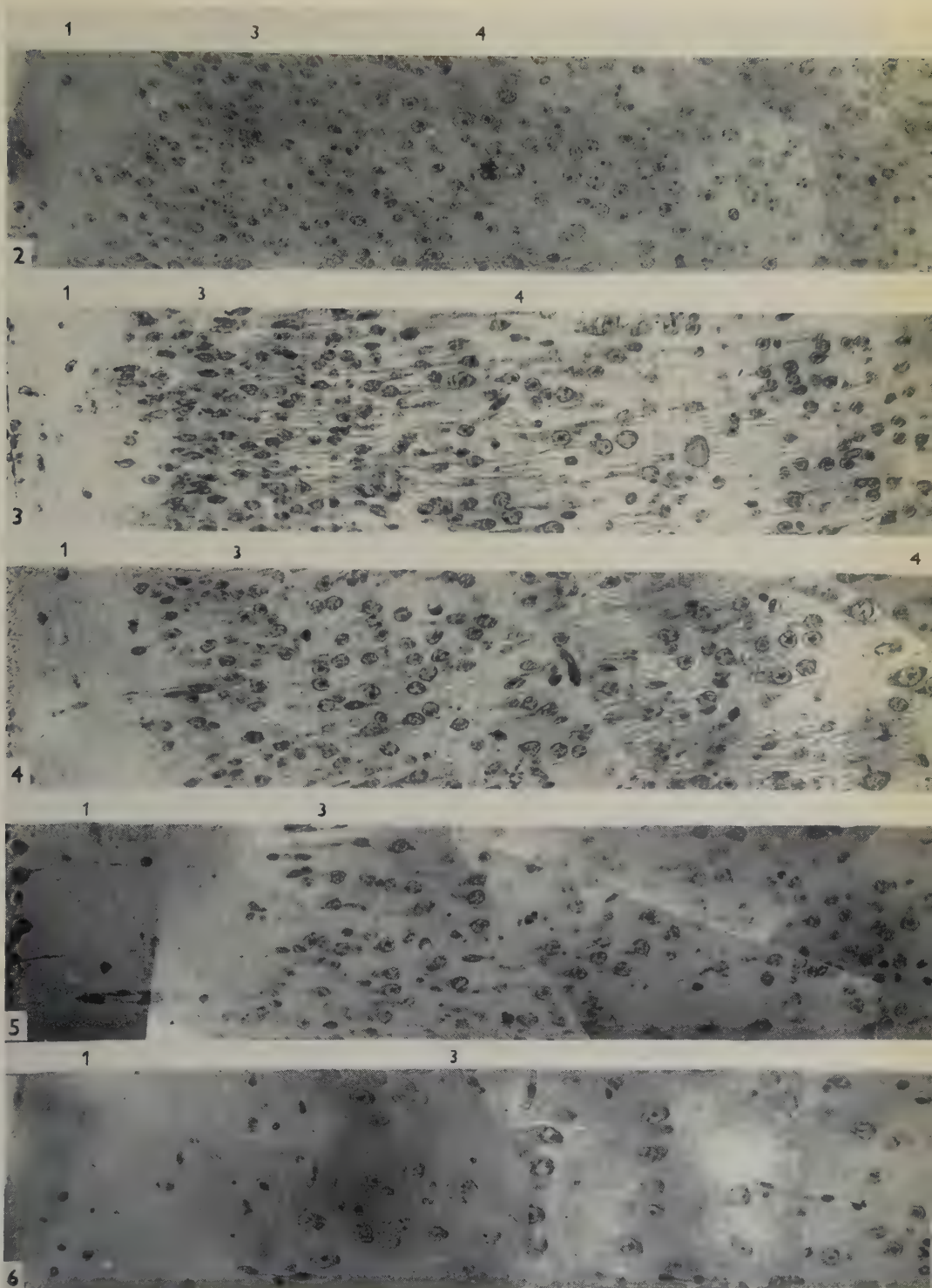
To test how far these conceptions of the growth of the developing nerve cell can be generally applied in neuro-embryology, a series of studies in the ultra-violet over a wide range of developing neurones will first be necessary. A point of importance in such inquiries will be how far the intervening period of minimum absorption varies in time with different neurones in the same embryo. In the chick (Hughes, unpublished) it appears that in those of the visceral autonomic system, the whole series of changes in absorption come at a much later period of development than in the spinal cord. Data of this kind may help to demarcate within the nervous system, what have been called by Streeter (1951) 'horizons of development' as well as to widen our knowledge of the cellular physiology of the developing neurone.

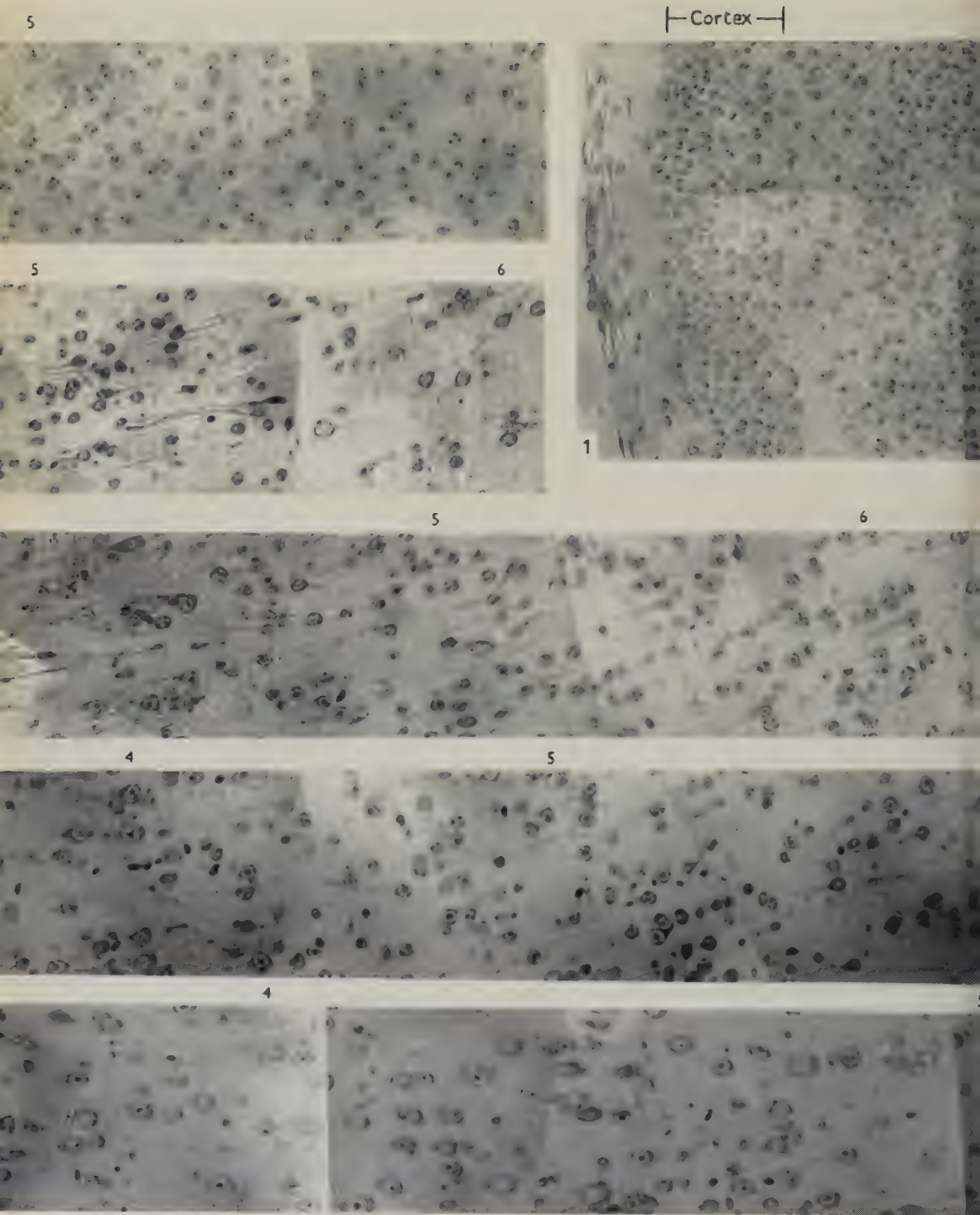
SUMMARY

1. The development of the cerebral cortex of the guinea-pig has been studied by means of the ultra-violet microscope.
2. The results have been compared with those previously obtained on the same material by biochemical and also by other histological methods.
3. Further evidence has been obtained on the changes in concentration of RNA during the development of the neurone.
4. This has strengthened the analogy already suggested between the development of the spinal cord of the chick embryo and the cerebral cortex of the guinea-pig. In the development of both, there are two periods of high RNA content, separated by an intervening minimum.
5. In both, cytoplasmic RNA is concentrated round the nuclear membrane at the earliest stage of differentiation of the neurone. By the time that the first peak is reached, this material has spread peripherally along the cell processes, from which it largely disappears during the subsequent minimum. RNA accumulates in the perikaryon once again in the second period of high concentration.

The expenses of that part of the investigation which has been made at Cambridge have been met from a Grant from the Nuffield Foundation to the School of Anatomy.







REFERENCES

- BRACHET, J. (1953). The use of basic dyes and ribonuclease for the cytochemical detection of ribonucleic acid. *Quart. J. micr. Sci.* **94**, 1-10.
- CASPERSSON, T. O. (1950). *Cell Growth and Cell Function*. New York: Norton.
- FLEXNER, J. B. & FLEXNER, L. B. (1951). Biochemical and physiological differentiation during morphogenesis. XIV. The nucleic acids of the developing cerebral cortex and liver of the foetal guinea-pig. *J. cell. Comp. Physiol.* **38**, 1-16.
- FLEXNER, L. B. (1952). The development of the cerebral cortex. A cytological, functional, and biochemical approach. *Harvey Lect.* **47**. New York: Academic Press.
- FLEXNER, L. B., TYLER, D. B. & GALLANT, L. J. (1950). Biochemical and physiological differentiation during morphogenesis. X. Onset of electrical activity in the developing cerebral cortex of the foetal guinea-pig. *J. Neurophysiol.* **13**, 427-430.
- FORTUYN, A. B. D. (1914). Cortical cell-lamination of the hemispheres of some rodents. *Arch. Neurol. Psychiat.* **6**, 221-354.
- HUGHES, A. F. (1955). Ultraviolet studies on the developing nervous system of the chick. Pp. 166-169 in *Biochemistry of the Developing Nervous System*. New York: Academic Press.
- HUGHES, A. F. (1955). The development of the neural tube of the chick embryo. A study with the ultraviolet microscope. *J. Embryol. exp. Morph.* **3**, 305-325.
- HYDÉN, H. (1943). Protein metabolism in the nerve cell during growth and function. *Acta physiol. Scand.* (6 Suppl.), **17**, 1-136.
- NOVIKOFF, A. B. & POTTER, V. R. (1948). Changes in nucleic acid concentration during the development of the chick embryo. *J. biol. Chem.* **173**, 233-238.
- PETERS, V. B. & FLEXNER, L. B. (1950). Biochemical and physiological differentiation during morphogenesis. VIII. Quantitative morphological studies on the developing cerebral cortex of the foetal guinea-pig. *Amer. J. Anat.* **86**, 133-161.
- STREETER, G. L. (1951). Developmental horizons in human embryos. *Contr. Embryol. Carneg. Instn.* no. 197.
- WILLIER, B. H. (1955). Ontogeny of endocrine correlation. In *Analysis of Development*, ed. Willier, Weiss & Hamburger. Philadelphia: Saunders.

EXPLANATION OF PLATES

Pls. 1 and 2 are of unstained sections through the cerebral cortex of the foetal guinea-pig, photographed at 2537A. Pl. 1 is at low power ($\times 340$ unreduced) and is so arranged that in each figure the outer surface of the hemisphere is to the left; the cortical layers are indicated by numbers at the top of each figure.

PLATE 1

- Fig. 1. 28 days p.c. The cortex is about 0.07 mm. thick, and occupies about one-quarter the width of the photograph. Compare with Pl. 2, fig. 7.
- Fig. 2. 33 days p.c. Cortex is about 0.7 mm. thick, and extends through the whole width of the photograph. Even at low magnification fibres are visible at all levels. Marked increase in size of some nuclei in layer (4).
- Fig. 3. 35 days p.c. Cortex now 0.9 mm. thick. Large ascending processes in layers (3) and (4) are conspicuous.
- Fig. 4. 40 days p.c. Cortex now 1.0 mm. thick. Ascending processes now evident in deeper layers.
- Fig. 5. 45 days p.c. Cortex 1.15 mm. thick. Layer (6) not included in photograph. Absorbing material disappearing from processes; at this time the first Nissl granules are recognizable in the perikarya.
- Fig. 6. Newborn. Cortex 1.45 mm. thick. Layer (6) and part of layer (5) not included in photograph. Ascending processes now show no more absorption than that of the surrounding neuropil. The cytoplasmic RNA is confined to the perikarya, and concentrated in the form of Nissl bodies.

PLATE 2

Figs. 7-11 are taken with a glycerin-immersion monochromat of 1.25 N.A. ($\times 1500$ unreduced). The molecular layer of the cortex is now towards the top of the plate in each figure.

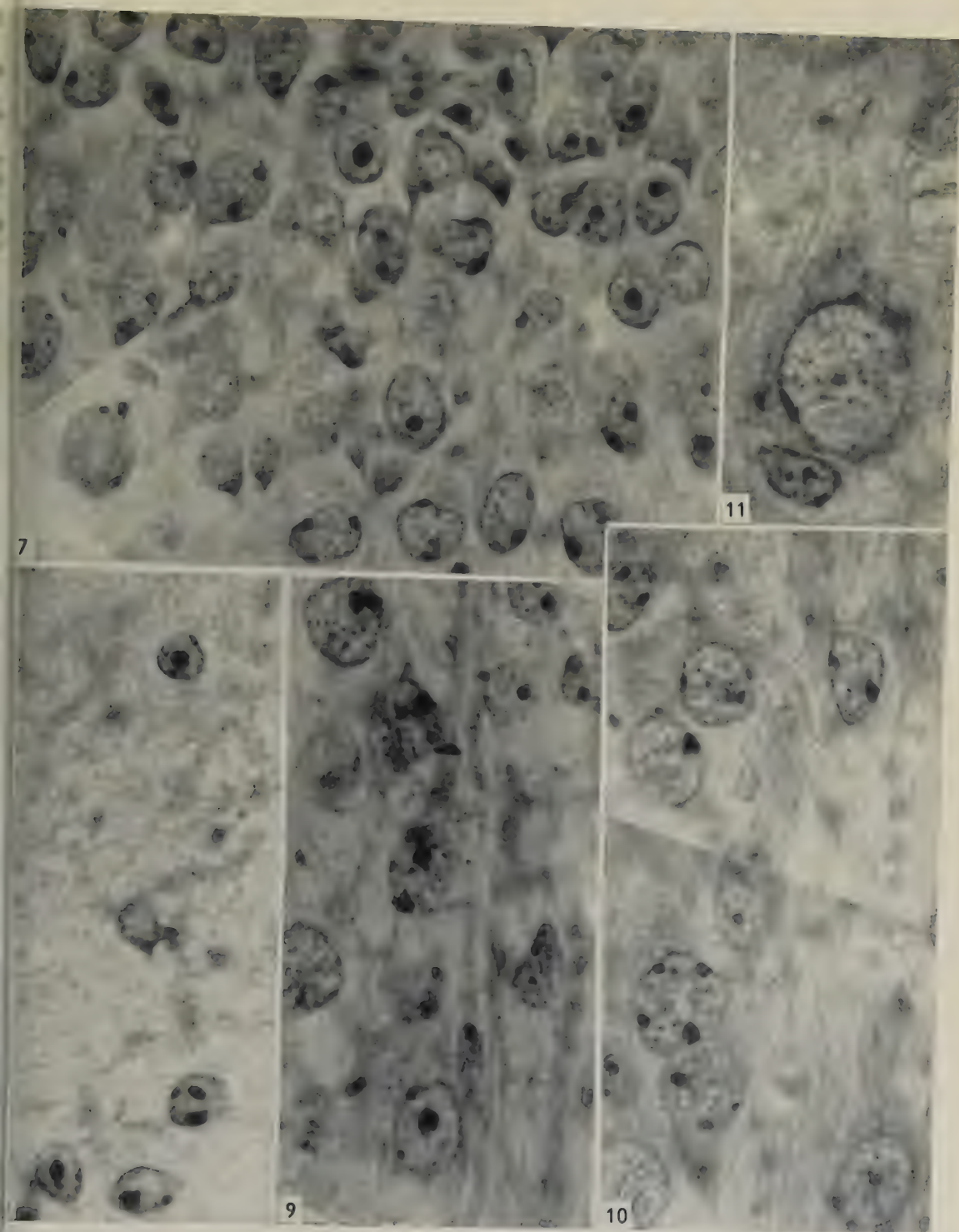
Fig. 7. 28 days p.c. This represents an enlarged view of part of Pl. 1, fig. 1. In some neuroblasts over one pole of the nucleus is dense material, which is continuous with a nerve process.

Fig. 8. 33 days p.c. Molecular layer, to show how distinct is each individual nerve fibre. This appearance is lost only in the last third of gestation.

Fig. 9. 35 days p.c. Cells from layer (4), showing the density of RNA in the ascending processes.

Fig. 10. 45 days p.c. Cells from layer (4). The contrast in the ascending processes is now much lower.

Fig. 11. Newborn. Neurones of layer (4). Ascending process shows no more absorption than that of the surrounding neuropil. The cytoplasmic RNA is confined to the perikarya, and concentrated in the form of Nissl bodies.



THE EARLY DEVELOPMENT OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)

By W. J. HAMILTON

Charing Cross Hospital Medical School, London

AND THE LATE D. M. SAMUEL

St Bartholomew's Hospital Medical College, London

INTRODUCTORY NOTE

The present investigations were begun by the late D. M. Samuel of St Bartholomew's Hospital Medical College in the summer of 1940. At the time of his death, in November 1943, he had collected a large number of eggs of this rodent at different stages of fertilization and cleavage, and had made many observations on the size of the living eggs. Before his death Samuel had prepared a manuscript on his findings. It has always been intended to complete the work, but only recently has the opportunity arisen to do so.

INTRODUCTION

The golden hamster (*Cricetus mesocricetus auratus*) is a small rodent, a native of Syria, and it was first introduced into this country by Dr S. Adler of the Microbiological Institute, Jerusalem. It breeds readily in captivity.

A striking feature of the golden hamster is the extreme shortness of its gestation period, this being only 16 days, or, in young vigorous females, often $15\frac{1}{2}$ days from the time of mating; occasionally it may be prolonged to $16\frac{1}{2}$ or 17 days (see also Bond, 1945). The young are able to fend for themselves after about a fortnight, practically as soon as their eyes open; the females can produce their first litter at an age of about 68 days, i.e. about 84 days after they themselves were still in the single-cell stage! The range in age of females for the first litter is from 59 to 90 days, with an average of 73.6 days (Bond, 1945; Graves, 1945). In our experience the breeding is not, however, so prolific as these figures might suggest.

The normal mating season is from February to September, but fertile matings are infrequent at the limits of this period (see Bond (1945) and Reed & Reed (1946) for details). Bruce & Hindle (1934) state that litters have been born during every month in the year. Deanesly (1938) suggested that the females were reproductive throughout the year, but that the males had a definite non-breeding season. Experiments with various hormones (Peczenik, 1942) have established that the animals can be made to breed during the winter, and this has been confirmed in our colony by using a single subcutaneous injection of 25–30 mg. of pregnant mare serum. The matings usually took place on the third or fourth day after the injection.

The oestrous cycle, as evidenced by cyclic changes in the vaginal smear, has been described in detail by Kupperman, Greenblatt & Hair (1944), Kent & Smith

(1945) and Ward (1946). The length of the cycle has been definitely established during the present investigation by the recurrence of oestrous receptivity every 4 days in the unmated female.

Accurate timing of insemination is very easy as the animals, which mate rarely in the daytime, do so readily at dusk (see also Bond (1945) and Graves (1945)). It is probable that a high proportion of matings occur within 2 hr. after sunset, so that it was found early on in this study that the method of examining the vaginal smear daily for spermatozoa gives remarkably consistent results as regards the time of fertilization. By testing females for receptivity at hourly intervals, oestrus in its strictest sense has been found to last 4 or 5 hr. after sunset.

Ovulation is spontaneous, and as many as ten ova have been recovered from a single uterine tube. It seems probable that copulation takes place in the majority of cases before ovulation occurs.

TECHNIQUE

The technique used for procuring the living eggs has already been described by us (Samuel & Hamilton, 1942).

The recovery of the eight-cell to blastocyst stages from the uterine horn is achieved by flushing the cavity with Locke's solution. Eight-cell stages and early morulae are readily obtained, but blastocysts are more difficult to dislodge. To obtain the latter the uterine horn is firmly ligatured at its distal end and then carefully dissected away from its mesentery. It is finally freed by cutting the horn immediately below the isthmus, and any blood present is washed off the mesenteric border. A glass pipette with rubber bulb was used to distend the horn forcibly with Locke's solution; this opened up the uterine recesses near the eggs where the mucous membrane was already tending to adhere. After this preliminary distension the ligature was cut away and the horn flushed straight through. Up to eight blastocysts have been obtained in this way from a single horn.

Eggs recovered from ovarian follicles, or within the first few hours after mating, require to have the corona cells removed before examination. Weak trypsin-Locke solution is used for this, care being taken not to allow the digestion to go too far, as in this case the zona pellucida may be injured. Usually, however, even in unfertilized eggs a perivitelline space is found to be present by the time the corona cells have been removed. As a rule 15 min. at room temperature is quite enough to loosen the cells of the corona so that they can be dislodged by gentle agitation, though at the earliest stages they are still very adherent.

Individual eggs are then pipetted through two changes of Locke's solution to remove debris, this procedure being carried out as rapidly as possible. The eggs are photographed in Locke's solution on a Vickers projection microscope which, having the optical system inverted, is well suited to this type of work. Photographs are taken at varying magnifications and at different levels in the egg. It was found that, in general, a low-power objective with a high secondary magnification gave the best general picture of the whole egg. Some photographs, to show detail within the unicellular eggs, have been procured by using a Leica camera adapted for microphotography. To obtain the most critical focus it is necessary to cover the egg with a cover-slip, when lenses of high power and fairly short working distance can be used. The most satisfactory method is to make a light vaseline ring on a

slide and to place the egg in a small drop of Locke's solution in the centre of this ring. A cover-slip is lowered gently on to the ring and then slowly depressed until contact is made with the drop. Further pressure enlarges the area of the drop and with practice the cover-slip may be lowered sufficiently to allow an oil-immersion lens to be used for the examination of the egg, which is just free from the cover-slip. This gives the most detailed photographs of the egg, but unfortunately the cover-slip cannot be removed without considerable risk of losing the egg; the manoeuvre has, however, often been carried out safely and Pl. 1, fig. 6, was obtained by this means.

FIXATION

Many different fixatives were employed, as some produced peculiar coarse striations in the cytoplasm of the egg. Bouin's fluid, Bouin-Allen (PFA 3), Zenker (with acetic acid and with formalin), Carnoy, Susa, Perenyi, formalin, formol saline and Flemming's solution were used, and dilutions of many of them in distilled water or saline were tried. The most useful fixatives, both for free eggs and for those in the uterine tube or horn, were a 50 % dilution of Zenker-acetic with distilled water, and a mixture of equal parts of Bouin-Allen (PFA 3) and 0.6 % chromic acid. Flemming's solution, with or without acetic acid, gave the least striated appearance to the cytoplasm. The appearances produced in eggs fixed in it are discussed later. Formalin-Locke mixtures were satisfactory for the cytoplasm when used alone but nuclear detail was lacking.

The zona pellucida was very easily dissolved in acid solutions, especially formol-acetic. Sometimes in a single uterine tube some eggs would have the zona destroyed, but in others it was practically normal. This was probably due to different rates of diffusion of the various constituents of the fixative.

Much time, labour and material was saved by the use of an agar gel as a transparent vehicle for free eggs during dehydration, embedding, etc. For details of the technique, with a discussion of its results and possibilities, see Samuel (1944). Almost all the illustrations of eggs up to the early blastocyst stage are of sections obtained in this way.

Dehydration of fixed material was carried out by gradual passage through the alcohols, usually with 5 % increments from 40 to 95 % spirit, and two changes of absolute alcohol. Methyl benzoate (with 1 % of celloidin added) was used for clearing, and the specimens were then transferred to benzene, benzene and wax, three changes of wax and embedded in wax (m.p. 56° C.).

Serial sections were cut at 7–8 μ , except in the case of the eggs in agar which were usually cut at from 3 to 7 μ .

UNFERTILIZED SINGLE-CELLED OVA

Living eggs

Eggs recovered from follicles. A few specimens were examined at this stage, and the mass of corona cell which was always present had first to be removed mechanically or otherwise (see p. 396). The eggs were examined under an oil-immersion lens by means of the method described earlier and photographs were also taken at lower magnifications.

The specimen illustrated in Pl. 1, fig. 1, was removed from a follicle of a young hamster which had had a sterile mating 92 hr. previously. This animal would probably have mated in a few hours; thus the egg was probably almost, if not quite, ready to be shed.

The zona pellucida is surrounded laterally by corona radiata cells, those on the upper and lower surfaces having been removed. The zona itself is homogeneous, showing no striations but its outer surface is irregular; the vitellus completely fills the intrazonal cavity. The vitellus is of a yellowish colour when fresh and can be seen in the photograph to be granular. There are no large opaque masses but many small adjacent dark and light areas. These are due to refractile granules of various sizes scattered irregularly throughout the cytoplasm, thus giving the egg a mottled appearance. Near the periphery there are one or two oval clearer areas, one of which may represent the position of the second polar spindle as there is no clear indication of a nucleus in this egg.

Eggs recovered from uterine tube. These are at first surrounded by corona cells and their appearance is essentially similar to that just described. The corona cells soon become detached from the zona pellucida, by which time the perivitelline space has appeared and the single polar body is clearly visible. In favourable specimens examined under high magnifications, there may be an indication of the position of the second polar spindle, whose axis at this stage is not arranged radially to the surface.

Sectioned unfertilized eggs

It is not proposed in this paper to discuss maturation, etc., but to give only a brief description of the ripe ova. The ova, in a large mass of corona cells, lie free in the follicle while the corona cells themselves are arranged in one or two layers around the ovum. Scattered around for a considerable distance are large numbers of corona cells, sparsely dispersed in an eosinophil coagulum. The ovum figured in Pl. 1, fig. 2, was from a hamster which had mated $4\frac{3}{4}$ hr. previously. Both ovaries had distended follicles and ovulation appeared to be imminent.

FERTILIZED SINGLE-CELL EGGS

This stage has been covered comprehensively; the earliest phases were found 4 hr. after copulation.

Before definite pronuclei are formed

Living. No detail of the cytoplasm of the egg could be observed until the corona radiata cells had been removed. In several of these eggs the sperm head and tail were seen embedded in the cytoplasm. The tail usually lies just below the vitelline membrane; it loses connexion with the head as soon as the latter begins to swell. The middle piece was not observed. A spindle of the second maturation division (second polar spindle) could sometimes be made out near the periphery. There is no striking difference in the appearance of the cytoplasm of these eggs and the ovarian eggs.

Fixed. When the eggs, embedded in agar, were examined after clearing in methyl benzoate more detail could be made out. The sperm head could definitely be seen, but the second polar spindle was only visible in favourable circumstances. This

was due to the striated and granular appearance of the cytoplasm, making the spindle and chromosomes much less obvious.

On section, the sperm head is identifiable but the tail is more difficult to demonstrate. However, it is well shown in Pl. 1, fig. 3, where it is partly outside the ovum while the sperm head lies definitely within the cytoplasm. In this egg the polar spindle is at the telophase, characteristic of unfertilized eggs. It is not favourably cut in the egg already illustrated, but a similar spindle is seen in Pl. 1, fig. 4. The spindle is small and makes an acute angle with the surface of the vitellus.

At a slightly later stage the sperm head swells but is still recognizable by its characteristic shape; the second polar body is beginning to separate, the chromatin lying at the poles of the spindle. The spindle shown in these sections is still not arranged radially.

PRONUCLEAR STAGE

Living. At the beginning of this stage the cytoplasm seems free from any nuclear material. Fertilization is only indicated by the presence of two polar bodies of which one is usually well formed and distinct while the other is more swollen and transparent and may only be represented by debris.

At a slightly later stage the pronuclei, still separated, are just visible in the living state (Pl. 1, fig. 5). They do not stand out sharply and no detail can be made out. Their probable size in the fresh state is shown in Pl. 1, fig. 6, which was photographed after allowing a cover-glass to compress the egg into a flattened disk. The two pronuclei and sperm tail are clearly seen.

As the pronuclei enlarge they become more and more visible in the fresh state, until just before fusion they make a striking picture. Three eggs with very obvious pronuclei of differing internal structure are shown in Pl. 2, figs. 7-9. In Pl. 2, fig. 7, there is a large centrally placed globule in both the male and female pronuclei. Pl. 2, fig. 8, however, shows pronuclei in which the globules are much smaller.

Pl. 2, fig. 9, was photographed with an oil-immersion lens. The pronuclei are very distinct and are just in contact with each other. Both are of about the same size (pronuclei in this stage are always very nearly spherical). The pronucleus on the right of the photograph shows four globules of medium size, while that on the left shows five smaller globules. There is no evidence as to which is the male or female pronucleus. The vitellus shows a definite division into two regions. The peripheral region is pale in colour with a few small black granules scattered throughout. Centrally there is an oval aggregation of these granules which are closely packed round the two pronuclei. The region appears to be connected by strands to the vitelline membrane. It must again be emphasized that these granules are in the main refractile rather than essentially dark. One polar body is visible in the perivitelline space. In other specimens the centrally situated mass of granules is still obvious, while the pronuclei show varying arrangements of the globules.

Fixed. In whole eggs embedded in agar and cleared it is possible to recognize even the smallest pronuclei, while the large pronuclei are easily seen. By orientating the egg (see Samuel, 1944) it is possible to show both pronuclei on the same section (Pl. 2, figs. 10, 11).

A section through two of the earliest pronuclei stages obtained during this study is illustrated in Pl. 2, fig. 10; this egg was obtained from a hamster killed 9 hr. after mating. The two pronuclei have quite different appearances, one (in the upper part of the figure) being approximately circular in outline and with basophil nucleoplasm containing a few blue-black granules of small size, while the other is slightly larger, oval in shape, and shows a slight irregularity of outline. It contains more numerous and larger granules which in some eggs, but not in all, are attached to the deep surface of the pronuclear membrane.

It is possible on focusing carefully to make out a structure resembling a sperm tail near the smaller pronucleus but the cytoplasmic precipitation throughout the vitellus renders this uncertain. However, study of other eggs showing similar pronuclei or swollen sperm heads definitely establishes that the larger, paler, pronucleus is the female and the smaller, darker pronucleus the male. The basophil nucleoplasm is seen in the swollen sperm head, and is not lost until the pronucleus has attained a larger size than that in Pl. 2, fig. 11.

The pronuclei enlarge rapidly and become more spherical so that by 12½ hr. after mating, the pronuclei are approaching one another (Pl. 2, fig. 12). Each shows a definite fine perinuclear membrane and a reticular formation of chromatin, which is more obvious in the pronucleus to the left of the figure. The nucleoplasm of this pronucleus is also slightly darker than that of its fellow and this suggests that it is the male. The dark staining, however, is not so obvious in the actual section as at the particular focus depicted in the figure, for the two pronuclei are not lying at identical levels. The size also is not shown correctly for, in fact, the largest diameters of these two pronuclei are approximately equal. Other eggs at this stage show comparable appearances, though some have larger globules within the pronuclei and some pronuclei lie nearer to each other. This variation probably depends upon the point of sperm entry relative to the point of expulsion of the second polar body; two pronuclei are very rarely found to be actually in contact with each other at this stage. Both polar bodies (one shown in the figure) are nearer to the paler pronucleus, but the significance of this relationship, if any, is doubtful.

Three pronuclei in a single cell have been found in four instances, two of which were found in a litter of eggs from hamster 130. These eggs are of valuable assistance in differentiating the male from the female pronucleus as it is possible that the condition is the result of the penetration of the vitellus by two sperms. Thus two similar pronuclei should be male and a single dissimilar pronucleus female. One of these eggs shows two slightly smaller and darker pronuclei, one of which lies in contact with a large, paler pronucleus (Pl. 2, fig. 13). The polar bodies, though at some distance from any of the pronuclei, are both nearer to this pale pronucleus which is suggested as being the female one.

At about 16 hr. after mating the cytoplasm round the pronuclei begins to show a finer granularity, and in this stage fixation is more uniform and fewer striations are apparent. Each pronucleus is surrounded by such a zone and, as the vesicular pronuclei approach one another, these cytoplasmic zones merge and become more extensive while the remaining cytoplasm becomes more irregularly striated than before. The contiguous surfaces of the two pronuclei now become flattened. Eventually

in the fixed egg with centrally placed pronuclei the cytoplasm, as in the living egg, is demarcated into a peripheral coarsely granular zone and an oval perinuclear finely granular zone. There is considerable variation, however, in the extent of these zones in different eggs (Pl. 2, figs. 11, 14). The globules within the pronuclei persist until a very late stage (Pl. 2, fig. 14), but all disappear just before the nuclear membrane breaks down. Thus there is a stage when the pronuclei are filled with many small dark masses of uniform size (Pl. 3, fig. 15). The pronuclear membrane disappears leaving a mass of chromosomes in the centre of the cell and a spindle forms immediately. This loss of the pronuclear membranes may not take place synchronously, so that in some eggs one pronucleus contains colourless nucleoplasm and chromosomes separating from spiral threads, while in the other the chromosomes are free.

FIRST CLEAVAGE SPINDLE STAGE

Living. At the end of the pronuclear stage the egg at first is still spherical and the central granular zone is still oval in outline, probably indicating the axis joining the two pronuclei which were previously present. Later, the egg becomes oval in outline and some indication of the spindle is visible, as an elongated clear central area with indications of fibres on each side (Pl. 3, fig. 16). When such a spindle is viewed 'end-on' it appears as a well-marked clear area in the middle of a circular mass of dark granules; the whole vitellus from this aspect also appears circular in outline. The opacity on the right side of the vitellus is due to the second polar body; remains of the first polar body are also found in the perivitelline space.

Fixed. The first cleavage spindle can be made out with varying clarity in agar preparations (Pl. 3, figs. 17, 18). Generally speaking, the later the phase the more distinct the picture. Pl. 3, fig. 17, just shows signs of a groove in the cytoplasm on one side of the cell, while in Pl. 3, fig. 18, division is almost completed. Quite appreciable detail of the spindles can be made out and the whole picture is very similar to that of the stained sections. The greater sharpness of definition is to be expected when the vitellus becomes oval, and still more when it becomes constricted, as there will then be much less overlying cytoplasm to obscure detail.

Twenty-one eggs containing first cleavage spindles were sectioned. These cover most of the phases of mitosis, but it has not been possible to determine the chromosomal number. The centrosomes have not been identified, the ends of the spindle being invisible during the anaphase. The four eggs of which sections are illustrated in Pl. 3, figs. 19–22, were all recovered from a single hamster (183). Anaphase and metaphase show no special features but the closing phases accompanying division of the cell (telophase) are well shown in Pl. 3, figs. 21, 22.

Egg no. 2 from this animal, of which a section is shown in Pl. 3, fig. 21, is oval and shows a very clear cytoplasmic division into two zones, an outer with irregular cytoplasm and an inner oval mass of finely granular material with a quite definite somewhat wavy outline. There is the beginning of a cytoplasmic constriction at the upper surface of the figure; this is not artificial for it is more marked in the photograph of the same egg in agar (Pl. 3, fig. 17), where the constriction is just discernible at the base. The centre of the cell is occupied by a spindle in the beginning of the telophase. The chromosomes have almost entirely reached the

ends of the spindle and form two masses capped on the side furthest from the spindle by a clear unstained area free from cytoplasmic granules.

The late telophase is seen in Pl. 3, fig. 22. Three eggs have been sectioned at this stage. Most of the features are similar to those just described, so attention will only be called to a few points. The cytoplasmic aggregation has an elongated narrow form and appears to be related more to the *cell* than to the nucleus. It seems that after division is complete the nuclei will be near the periphery of the aggregation (cf. description of early two-cell). The nuclei are oval both in the agar gell and in the final preparation, so each must have the shape of an ellipsoid of revolution round the smaller axis. The constriction of the cytoplasm has advanced considerably; this process was taking place *in vitro* at room temperature and was arrested by fixation.

TWO-CELL STAGE

Living. As this stage extends over a long period (about 25 hr.) many two-cell eggs have been recovered and photographed (Pl. 4, figs. 23, 24).

Division is usually not quite equal, and there may be great disparity between the blastomeres. As all gradations occur it is difficult to suggest the point at which mere inequality becomes abnormality. An extreme case which is undoubtedly abnormal, even though a nucleus is present in each blastomere, is shown in Pl. 4, fig. 25.

The earliest two-cell stages (24–26 hr. after mating) show a flattened contact area of variable extent in each blastomere (Pl. 4, fig. 23). When the blastomeres are viewed 'end-on', i.e. superimposed, their outlines are almost exactly circular, so that the general form of each is that of an ellipsoid of revolution with one face flattened. The zona pellucida is often ellipsoidal due to the pressure of the blastomeres, and the perivitelline space is seen to contain polar bodies or remains of these. The cytoplasmic aggregation so characteristic of the later single-cell stages is still very evident and it quite frequently extends to the surface of the blastomere at some point near, but not necessarily at, the contact area. The remainder of the cytoplasm is similar to that described for the single-cell stage.

Two-cell eggs, as in Pl. 4, fig. 23, show nuclei which are circular and well defined and contain numerous small granules or globules. They lie in the cytoplasmic aggregation but apparently bear no definite relation to it as they may be found at one extremity of the aggregation and, indeed, may be very eccentrically placed in the blastomere as a whole.

Two-cell eggs (about 40 hr. after mating) show, as a rule, only a very small contact area, the blastomeres being now nearly ellipsoidal (Pl. 4, fig. 24). The nuclei are approximately centrally placed and though they are still surrounded by a dark granular aggregation it is neither so uniform nor so definite as immediately after division of the single-cell.

The location of the sperm tail in the single-cell egg is apparently fortuitous and the tail may still sometimes be seen at the two-cell stage. It often passes from one blastomere to the other across the contact area, but it may bridge the inter-cellular sulcus as in Pl. 4, fig. 24.

Fixed. The great difficulty in this and succeeding cleavage stages was to secure fixation without producing the artificial appearance described earlier. The two-

cell stages were less troublesome in this respect than eggs with more blastomeres, but the large variety of fixatives used is responsible for the differing appearances in the final preparations figured. Pl. 4, figs. 26 and 27, are sections of eggs which were obtained from females only 24½ and 25 hr. after mating, and they are most unlikely to be more than 2 or 3 hr. after the first cleavage. The fixative in these eggs was Bouin-Allen (PFA3) diluted with an equal quantity of distilled water, but the microscopic appearances are very different. The specimen illustrated in Pl. 4, fig. 27, and fixed in the uterine tube, shows the central cytoplasmic aggregation surrounded by a more peripheral zone which has the same artificially-produced fragmented appearance seen at the first cleavage spindle stage. The specimen illustrated in Pl. 4, fig. 26, was fixed after removal from the tube and was then sectioned in agar; it shows a very different cytoplasmic picture.

The general appearance of the cells changes remarkably little during the 24 hr. or more before the next cleavage and, as the cytoplasm is similar in the early and late phases of the two-cell stage, only the latter will be described in detail. It will be seen, however, that the nuclei at the earliest two-cell stage (Pl. 4, fig. 26) have already swollen, losing the clear unstained area which surrounded the chromatin masses which were their immediate precursors. There is now a distinct nuclear membrane and the homogeneous blue-black mass of chromatin has given place to a paler nucleoplasm with threads and globules characteristic of mature nuclei. The central cytoplasmic aggregation reaches the surface of the blastomere at one or more points, as is indicated by the photographs of eggs in the fresh state. The nuclei do not bear any fixed relationship to the cytoplasmic mass, often being eccentrically placed at first but, as a rule, becoming more central as they enlarge.

The later period of the two-cell stage is represented in Pl. 4, fig. 28, 49 hr. after mating, three of the six other eggs of the litter showing mitotic figures marking the end of the stage. This specimen was also obtained during experimentation with various fixatives and was fixed in 10% formalin for 1 hr. followed by 10% Zenker-acetic (in distilled water) overnight. The fixation is very good as regards the structure of the cytoplasm, but the cells have swollen slightly and then been secondarily compressed by the zona pellucida, thus giving no true picture of their original shapes as seen in photographs of eggs in the fresh state.

The cytoplasm is finely granular, both in the peripheral and central areas, but the central aggregation is still clearly defined by its definite dark colour. Some of this darker granular material is drawn out into strands which pass from the central mass into the paler peripheral area, sometimes reaching the vitelline membrane, where it is continuous with a narrow zone of darker material, visible at most parts of the circumferences of the blastomeres. The nuclei are much larger than in the early two-cell stage but are not spherical, especially that of the blastomere to the right of the photograph; this nucleus has an irregular shape though with a smooth surface. The other nucleus is approximately circular in outline. Both nuclei contain many irregular blue-black granules which may be grouped into three classes: (1) a fine, central, reticular formation; (2) medium-sized granules mostly lying close to the nuclear membrane; and (3) larger dark masses placed in different regions. The appearance of the cytoplasm resembles very closely indeed the cytoplasmic appearance of unfixed eggs.

Volumetric differences between the blastomeres vary from approximate equality through all gradations to the obvious disparity shown in Pl. 4, fig. 29. It is interesting to note that the nucleus in the smaller cell is double, or lobed to an extreme degree, a feature which was shown by both nuclei in each of three young two-cell eggs from hamster 155. There is usually no detectable qualitative difference between the blastomeres, but this will be discussed later.

THREE- AND FOUR-CELL STAGE

Living. Only two three-cell stage eggs were recovered and there was nothing in them worthy of special note while in the fresh state, the larger blastomere dividing before the smaller. Four-cell eggs are shown in Pl. 4, fig. 30, and Pl. 5, fig. 31. The enlargement of the intrazonal cavity and the total volumetric shrinkage of the vitelli allows the blastomeres some freedom of movement. They are usually arranged in the tetrahedral form depicted in Pl. 4, fig. 30, but may show various arrangements between this state and that shown in Pl. 5, fig. 31, where they all lie approximately in the same plane. It must be pointed out, however, that four cells, in the form of a pyramid, may each be touching the zona, and yet a photograph may show a clear perivitelline space surrounding them, depending upon the level which is in focus.

Contact areas are usually small but occasionally may be extensive. The cytoplasm shows the typical appearance found in earlier stages, but larger granules are seen in the perinuclear region. Nuclei can sometimes be observed but often are only indicated by this cytoplasmic aggregation. One or two polar bodies are still visible.

Fixed. All the evidence points to the fact that in the hamster, as in most mammals so far studied, the blastomeres divide in a cruciform manner, i.e. the spindles of division are at right-angles to each other, giving rise to the tetrahedral arrangement of the cells as described in the living egg. The larger cell probably divides earlier, but sometimes both cells divide almost simultaneously. The larger cell of the two-cell stage shown in Pl. 5, fig. 32, has already divided giving rise to two daughter cells, one of which is seen in the lower part of the photograph. The other daughter cell lies immediately behind this in the succeeding sections so that a division of the remaining cell (obviously the smaller of the first pair) must inevitably give rise to a tetrahedral blastomeric arrangement. The spindle of division in this case has the same general appearance as that of the spindle of first cleavage, except that the equatorial plate does not seem so extensive in this cell. The central cytoplasmic aggregation is still discernible though not so obvious in this particular preparation. In the daughter cell the immature nucleus lies eccentrically towards the upper right side and is rather indistinct in this section. The nucleus of the remaining daughter cell, in a succeeding section of the series, shows a very similar appearance, with the chromatin arranged in many small dark masses of equal size without any distinct nuclear membrane. Each of these nuclei is surrounded by a cytoplasmic aggregation similar in appearance to that of the two-cell stage, but much less in quantity. As in the early two-cell stage, it is noticeable that the recently formed nucleus lies eccentrically not only in the whole blastomere but in

the cytoplasmic aggregation; also, just below this nucleus, there is a small palely staining zone very like the clear 'cap' seen at the ends of the spindle.

In many of the eggs at the four-cell stage, which were obtained 60 hr. after mating, the typical pyramidal arrangement is shown. Only in rare cases do the four cells lie in a single plane and advantage was taken of this arrangement to secure the section shown in Pl. 5, fig. 33, which was obtained by orientating the egg in agar.

In some eggs the cytoplasm shows the fragmented appearance already mentioned, but in varying degree according to the method of fixation. Most of the four- and eight-cell eggs sectioned in the uterine tubes were almost valueless cytologically for this reason. One or two polar bodies, inconstant in position, may still be identified, and in some eggs debris is present in the perivitelline space.

FIVE- TO EIGHT-CELL STAGE

Living. Eggs at the five-cell stage have been found on several occasions; they show in each case one large cell and four smaller cells (Pl. 5, fig. 34). Obviously these eggs had two large and two small cells in the four-cell stage and one of the larger cells has been the first to divide.

Eggs at the six-cell stage are often difficult to interpret; one is shown in Pl. 5, fig. 35. It appears that the two sharply focused cells are the remaining cells of the four-cell generation. However, an examination of Pl. 5, fig. 36, the same egg at a different focus, makes this interpretation doubtful. At the seven-cell stage similar difficulties are encountered.

Eggs at the eight-cell stage are recoverable either from the uterine tube or the uterus; until about 72 hr. after copulation the blastomeres are typically spherical with small contact areas (Pl. 5, fig. 37). The perivitelline space is usually extensive and, as a rule, only one polar body is visible.

The arrangement of the eight cells is usually difficult to interpret, but in the later stages of this study it was found that the blastomeres of the early eight-cell stage usually lie as in Pl. 5, fig. 37. It will be readily understood that if such an egg were viewed from almost any other aspect this arrangement would be masked in the photograph, but it has been found that if eggs are rotated while under microscopic observation this disposition of the cells can be made out. Sometimes four large and four small cells can be distinguished, but usually the differences are less obvious. The problem of cellular arrangement is further discussed under cell lineage.

The granules seen in the cytoplasm are fewer in number than in earlier stages, the cells often appearing empty. Occasionally larger dark granules are present, usually in the vicinity of the nucleus, where the perinuclear cytoplasmic aggregation is much less extensive than in the two- and four-cell stages. The nuclei themselves lie peripherally in the blastomeres, an arrangement seen much more clearly in the cleared eggs in agar.

About 70-72 hr. after mating the blastomeres rapidly change their characters and lose their spherical form. The eight cells become one solid mass having the rounded outlines of individual cells on its surface. An early phase of this condition, which merges into the morula stage, is shown in Pl. 6, fig. 38.

Fixed. Few new observations have been made of stages between four and eight cells. The sections of the six-cell egg illustrated in the fresh state in Pl. 5, fig. 35, are difficult to interpret as all the nuclei are in the resting phase.

An eight-cell stage egg photographed in agar is shown in Pl. 6, fig. 39, and the peripheral situation of the nuclei, which was occasionally suggested in the unfixed egg, is now definitely established. Sections of an eight-cell egg are shown in Pl. 6, figs. 40-43. They are the second, third, fourth and seventh of a series of eight sections, of 7μ thickness, and from them the blastomeric arrangement may be determined. The cells form a compact mass, with clearly defined cell-membranes, though it is not possible to state the degree to which compression by the zona during or after fixation is responsible for the compact arrangement, as no photograph of this egg in the living state was secured. There are only small differences between the cells, and it is possible that the two nucleated cells in Pl. 6, fig. 42, are destined to become trophoblast cells and to overgrow the remaining cells. This process of overgrowth is much more definite in the morula-blastocyst stage. The cytoplasm of all the cells shows only slight striations. Two cells appear slightly lighter than the remaining cells, and this may point to the likelihood of their being destined for cells of the inner cell mass. There is also the definite evidence of organization provided by the fact that the nuclei are situated peripherally, not only in each cell but in relation to the morula as a whole. The nuclei are slightly ellipsoidal in shape, with the long axes of the ellipsoid parallel to the cell surface and the short axes arranged radially. Thus the nuclei cut in terminal sections (as in Pl. 6, fig. 40) appear circular in outline. There are no obvious differences in nuclear size or structure, many small and a few dark chromatin masses being apparent.

MORULA AND BLASTOCYST STAGES

In this animal at least, there is no justification for separate descriptions of these stages, as the blastocyst stage may indeed precede the formation of a morula in the generally accepted sense. This is shown in Pl. 6, fig. 44, where an eight-cell blastocyst is illustrated.

Living. Blastocyst formation has been observed as early as $69\frac{1}{2}$ hr. after mating, in one case when there were definitely only eight cells and in another where there were either eight or nine cells (Pl. 6, fig. 45). A polar body may be seen outside the mass, and sometimes one of the cells seems to have been excluded in the organizing process and lies to one side of the morula (Pl. 7, fig. 46).

Blastocyst formation, in the golden hamster, begins by the sudden accumulation of fluid between two cells and may be at first at the expense of a single cell which becomes indented. This is definitely so in Pl. 6, fig. 44, the eight-cell blastocyst in which one cell has become crescentic owing to the formation of a globular cavity. Pl. 6, fig. 45, shows a similar but larger cavity in an eight- or nine-cell blastocyst with at least two cells indented. It seems most unlikely that either of these two blastocysts had an inner cell mass, as, in the first specimen at least, the typical eight-cell arrangement of the blastomeres is evident. The formation of the cavity is not always so early and frequently a solid cellular morula is first formed. The blastocyst cavity, when it appears, separates the trophoblast cells from an inner cell mass. This cavity is at first irregular, but later takes up a hemispherical form

with more and more cells in the thinned-out portion. At first there are only one or two cells stretched over the cavity (Pl. 7, fig. 47), but in the later stages there may be six or more cells involved and the surface of the inner cell mass nearer to the cavity becomes remarkably flat (Pl. 7, figs. 48, 49). The cytoplasm in the morula and blastocyst stages does not differ markedly from that of earlier stages; but aggregations of dark and light granules are less distinct. Nuclei are only occasionally visible.

The zona pellucida usually shows changes within a few hours of the egg entering the uterus. Sometimes the intrazonal cavity is greatly expanded and the outer surface of the zona can scarcely be identified, so that the thickness of the zona is very difficult to gauge in these specimens.

Fixed. The earliest stage in the formation of the blastocyst proper is shown in Pl. 7, figs. 50–54, which are serial sections of a thickness of 7μ through the central part of the egg. There is now a definite distinction between the covering trophoblast cells and the inner cell mass.

The cells of the trophoblast are more darkly staining than those of the inner cell mass, and though both show the striated appearances characteristic of the cytoplasm of eggs fixed in earlier cleavage stages, the condition is more marked in the central cells.

The nuclei of the trophoblast cells are large and almost spherical; they are placed centrally in the widest part of the flattened cells. The nuclei of the cells of the inner cell mass are slightly smaller. They retain, however, a tendency to be situated in the more peripheral part of the cell. It is possible to find differences in the staining properties of certain cells, but these do not seem to be constant and have not been correlated with further developmental potentialities.

The next phase in development is characterized by increase of the blastocyst cavity. This is usually accompanied at first by stretching out and attenuation of the two or three trophoblast cells bounding the cavity, but later more cells are present. In the blastocyst illustrated in Pl. 8, figs. 55–60, at least six and possibly seven trophoblast cells line the cavity; in this specimen there is a definite trophoblastic layer—attenuated but still definite—which covers the inner cell mass. The cytoplasm of the trophoblast cells is slightly more deeply stained than that of the inner cell mass, though the nuclei show no additional characters to those noted in the earlier stage. The cells of this inner cell mass do not differ from one another but the peripheral cells seem to have a tendency to separate from the trophoblast cells at the edge of the cavity (Pl. 8, fig. 57), which may be in order to allow the trophoblast cells to migrate to add to the wall of the cavity.

This is almost the latest stage which can be successfully obtained by uterine flushing, for even though a few blastocysts, in which the zona pellucida is absent, have been obtained, they show no significant differences. Thus subsequent stages which show an increasing degree of attachment to the mucosal pocket in which they lie had to be sectioned *in situ*, entailing the use of many animals in order to obtain satisfactory specimens.

The difficulty found in sectioning these attaching blastocysts is due in very great measure to the irregular orientation of the blastocyst within the horn. It lies always antimesometrially but the relation of the inner cell mass to the axes of

the uterine horn is very variable indeed, varying from 'typical' cases in which the blastocyst is orientated with its inner cell mass mesometrially placed through every gradation to an antimesometrial position. This variability continues until the latest stages considered in this present study, but the later the stage, the more likely is the inner cell mass to be situated mesometrially in relation to the blastocyst as a whole. The appearance of the pocket in which these early blastocysts lie may not be representative of the condition in the living state. There is no doubt that, during fixation, tissues shrink and that this shrinkage first affects the peripheral layers of the uterine horn before reaching the endometrium. Thus the outer layers are contracting upon the inner unfixed part and by the time the innermost tissues are being fixed (and so shrinking) in their turn, the outer layers are relatively rigid. This will result in fluid passing into the lumen, resulting in artificial distension. To a greater or less extent this process is continued through the various stages of dehydration, embedding, etc. Local physical conditions probably play a decisive role in the extent of this process, for not all the blastocyst pockets in the same horn may show the same distortion. The constitution of the fixative is also important, and in one case a series of blastocysts was sectioned in which no artificial distension of the cavity had occurred.

The first sign of definite attachment of the blastocyst is shown in Pl. 8, fig. 61. The whole blastocyst is roughly ellipsoidal in shape. The trophoblast is most strikingly altered from the layer of flattened cells characteristic of earlier stages. The abembryonic cells swell first and exhibit a paler cytoplasm; this change takes place to a lesser degree over about two-thirds of the circumference of the blastocyst. The nuclei begin to increase in size and become more spherical, and this trophoblastic pole often gives at first sight the appearance of another inner cell mass. The cells comprising the remainder of the trophoblast, i.e. the part related to the inner cell mass, and extending just beyond it, remain flattened. In some specimens it is extremely difficult to decide whether there are persistent trophoblastic cells covering the inner cell mass.

In other specimens the inner cell mass occupies only a small portion of the whole blastocyst and consists of about seven to ten cells which are darkly staining and with clearly defined oval nuclei, usually in the resting phase. At this stage it is not possible to differentiate the primordial endoderm cells, if indeed they exist.

The next stage of development in our series is illustrated in Pl. 8, fig. 62, from a hamster killed 109 hr. after mating. It shows a somewhat collapsed blastocyst. The trophoblastic cells are more swollen and palely staining than before and their boundaries have become indistinct, especially on their inner surfaces, near to the blastocyst cavity. Definite invasion of the mucosa has taken place and is especially marked at a few points where processes of the trophoblast cells penetrate deeply. Changes in the mucosal cells do not come within the scope of this study. The inner cell mass shows a distinct segregation into primordial endodermal and ectodermal cells. The endoderm cells have more darkly staining nuclei and the cells nearest the trophoblast have processes which seem to be spreading round on its inner surface. The appearances seen in Pl. 8, fig. 62, suggest that the endoderm cells are excalated from the inner cell mass and take up their position next to the blastocyst cavity.

The formative cells, more numerous than the endoderm cells, form a compact mass covered by a thin trophoblast layer and their nuclei are larger and paler than those of the endoderm layer.

The presence or absence of 'parietal endodermal' cells cannot be determined from the photographs nor from the sections as the trophoblast layer is folded.

VOLUME OF THE VITELLUS AT DIFFERENT STAGES OF DEVELOPMENT

By making measurements on photographs of a number of living eggs at different stages of development a comprehensive series of measurements has been obtained (see Table 1).

For the unsegmented egg the average volume of the vitellus of twenty-six eggs is $253,600\mu^3$. The smallest vitellus had a volume of $313,000\mu^3$, the largest $377,000\mu^3$. The average diameter of the vitellus was 78.5μ . The percentage of total volume of the zonal cavity occupied by the vitellus is 55.

The average volume for the two-cell stage is $193,475\mu^3$; the smallest egg had a volume of $148,400\mu^3$ and the largest a volume of $280,500\mu^3$. The percentage of the total volume of the zonal cavity occupied by the vitellus is 39.9.

Table 1. *Averages for various stages*

	No. of eggs	Range of age (hr.)	Volume of vitelli (μ^3)	Volume of zonal cavity (μ^3)	Thick-ness of zona pellucida (μ)	Percent- age of total volume occupied by vitelli (μ^3)	Diameters of eggs	
							Internal	External
Unsegmented stage	26	$12\frac{3}{4}$ – $24\frac{1}{4}$	253,600	462,692	11.6	55	96	119
Two-cell stage	24	$25\frac{1}{2}$ –46	193,475	493,312	11.46	39.9	98	120.8
Four-cell stage	16	59–64	172,312	579,437	11.3	30.3	104.2	126.8
Eight-cell stage	13	60– $78\frac{3}{4}$	171,950	513,192	10.84	33.6	99.3	120.98
Blastocyst stage								
Whole blastocyst	4	79	185,175	512,250	9.1	36.8	99.5	117.7
Cytoplasm only	4	79	159,475	—	—	31.5	—	—

The detailed measurements of the volumes of all the eggs and of the different blastomeres up to the eight-cell stage are available in the Department of Anatomy, Charing Cross Hospital Medical School.

At the four-cell stage sixteen eggs were measured. The average volume of the vitelli was $172,312\mu^3$; the smallest vitelli occupied $145,000\mu^3$ and the largest $199,500\mu^3$. The percentage of the total volume of the zonal cavity occupied by the vitelli is now only 30.3. The total volumes of the blastomeres at the eight-cell stage is shown in Table 1. The smallest combined volume of the blastomeres is $160,500\mu^3$ and the greatest $186,550\mu^3$. The average volume is $171,950\mu^3$. The average percentage of the combined volume of the blastomeres occupying the zonal space is 33.6. In Table 1 the volume of the total blastocyst and the mean volume of the cytoplasm is given for four eggs. The average percentage of the zonal space occupied by the cytoplasm is now 31.5.

From the above figures it will be realized that there is a marked loss in the volume of the egg from the time of ovulation until the blastocyst is formed.

AGE, STAGE AND LOCATION OF EGGS

In the present investigation the uterine tube was divided into three segments of approximately 1 cm. each.; the location of the eggs is accurate only to this extent. Ovulation normally occurs 5 or 6 hr. after copulation. The pronuclei, formed about 9 hr. after copulation, grow and approach one another until at about 22 hr. they are vesicular and lie adjacent to each other. Division into the two-cell stage takes place between 23 and 28 hr. (usually 25 hr.) after copulation. This gives the one-cell stage a duration of about 20 hr. The two-cell stage lasts approximately until 50 hr. after mating, i.e. a duration of about 25 hr. From 50 to 62 hr. the egg is at the three- or four-cell stage and from 62 to 70 hr. it has reached the five- to eight-cell stage. At about 70 hr. after mating a morula is formed; the blastocyst stage may follow immediately or after only a few hours. A remarkable feature of the development of this animal is the fact that the two-cell stage should occupy a full day out of a total of just less than 16 days gestation.

The duration of the stages after ovulation is approximately: one-cell, 20 hr.; two-cell, 25 hr.; four-cell, 12 hr.; eight-cell, 8 hr.

It should be pointed out that the above figures are not exact but are reasonable deductions from data which, on the whole, are remarkably consistent. The most likely source of error would be a variation in the interval between copulation and ovulation in individual animals. For the evening-mated animals, however, it is probably from 4 to 7 hr., and 4 hr. have been subtracted from the time of copulation to the close of the one-cell stage to give the true length of that stage, i.e. 20 hr. It is admitted that this is but an estimation, but the durations of the later stages are more exact for the one-cell stage ends with remarkable constancy within 1 hr. either way of 24 hr. after copulation, thus giving a good basis for subsequent calculations. In fact between 23 and 25½ hr. after copulation, from nine animals killed, at least one egg from each animal was found to be at the stage of the spindle of first cleavage, and of the fifty-seven eggs recovered, twenty-two showed vesicular pronuclei, twelve were in the two-cell stage and no fewer than twenty-three were in the cleavage spindle stage.

COMMENTS AND COMPARISONS

Rates of cleavage and duration of each stage

The rates of cleavage for a number of different species have been summarized in a table by Hamilton & Laing (1946). When the rates of cleavage of the hamster in the present investigation are compared with those of Graves (1945), Venable (1946a) and Ward (1948) the times of the appearance of the different stages and their durations are remarkably constant. Graves found that ovulation occurred 6 hr. after copulation, and the two-cell stage lasted from 24 to 48 hr.; our times for the two-cell stage are from 25 to 50 hr. The egg reached the uterus as an eight-cell stage or an early morula by 70 hr. after copulation, and the blastocyst was formed shortly after 70 hr. Graves, Venable and Ward all found the eight-cell stage or morula at from 2½ to 3 days.

When comparisons are made with the rates of cleavage of other Muridae, it is found that the unsegmented egg persists up to about 24 hr. for the mouse (Lewis

& Wright, 1935; Sobotta, 1924), and for the rat (Huber, 1915). Gilchrist & Pincus (1932) found that the unsegmented egg persisted for 27 hr. in the rat. In the guinea-pig (Squier, 1932) the unsegmented egg was found as early as 3 hr. after copulation and persisted up to 30 hr. These differences may be accounted for by variable intervals between ovulation and copulation.

The two-cell stage in the hamster persists longer than in either mouse, rat or guinea-pig, although the gestation period is shorter than in any of these animals.

The five- to eight-cell stage is reached in the hamster 62–70 hr. after copulation, which is again later than for the corresponding times in the mouse as determined by Lewis & Wright (50–64 hr. for the eight-cell stage), but earlier than that found in the rat by Huber (89 hr.), Gilchrist & Pincus (71–95 hr.) and Macdonald & Long (1934) (64–89 hr.). The eight-cell stage in the guinea-pig lasted up to 80–85 hr. (Squier).

No reasonable explanation is forthcoming at present for different rates of cleavage in rodent eggs. With the exception of that of the guinea-pig, the eggs of these are essentially similar in size and appearance. Assistance from the study of the eggs of other mammals is also disappointing. Experiments in the culture of eggs *in vitro* may help in the solution of the problem. The account of Lewis & Hartman (1933) on the macaque shows the average duration of the one-cell, two-cell, three- to four-cell and five- to eight-cell stages to be 24, 12, 12 and 24 hr. respectively. It may reasonably be objected that there are many possibilities of error owing to the artificial environment of the eggs, but Lewis & Gregory (1929) culturing rabbit eggs *in vitro* found that the rates of cleavage were within the limits of *in vivo* development.

Size of the living mammalian egg

Measurements of recently ovulated living mammalian eggs have shown that there are considerable variations in their size. As long ago as 1929 Hartman reviewed the then available information on this subject and, apart from the monotremes and marsupials, showed that the mammalian eggs have a vitelline diameter from 70 to 140 μ . More recent measurements by Lewis & Wright (1935) for the mouse, and Amoroso, Griffiths & Hamilton (1942) for the sheep, have shown that the average size is 87.8 μ in the former animal and 153 μ in the latter as estimated by the inside measurement of the zona pellucida. It should be noted, however, that this difference is an eightfold volumetric variation. Very small eggs (diameter of vitellus 70–80 μ) are mainly confined to rodents. Table 5 of Lewis and Wright gives comparative sizes of some of the eggs within this group.

Of the eggs of the four members of the Muridae examined that of the guinea-pig is about the same size as that of the hamster but shows obvious cytoplasmic differences. Graves (1945) and Venable (1946*b*) give measurements for the fixed and sectioned eggs. Their measurements, as is to be expected, are much less than those of the living egg.

Appearance of the vitellus in the eggs of different species

The cytoplasm of the living mammalian egg shows marked differences in appearance in different species and even within a single genus. The differences are chiefly

due to the number of fatty globules present in the vitellus. When many globules are present most of the intracellular detail is obscured. These globules, in general, form a source of nutriment in those animals in which implantation does not occur for a relatively long time after the blastocyst has reached the uterus. The above relationship, however, is not always constant and there are a number of instances where there is no definite explanation (except presumably a teleological one) for the abundance or scarcity of the fatty globules. The eggs of the Muridae so far examined have a clear cytoplasm which makes them especially suitable for examination in the living state. In the hamster the nucleus is clearly seen. When comparison is made between the living hamster egg and that of the mouse (as described by Lewis & Wright, 1935), it is found that at the single-cell stage of the latter there are small, discrete yolk masses which give the vitellus a flecked appearance and obscure the details of nuclear structure. On the other hand, there is nothing comparable in the mouse egg to the arrangement of the cytoplasm in the hamster, both in the living and fixed egg at the pronuclear stage. In the hamster egg the cytoplasm shows a division into a peripheral, paler region and a central more granular region around the pronuclei or in later stages around the nucleus. This separation of the cytoplasm is found in the blastomeres of the two-cell stage and not so markedly in the later stages (up to the eight-cell stage). A similar separation of the cytoplasm into regions is described by Lewis & Hartman (1933) immediately following cleavage in the egg of the macaque monkey. They, however, found that the separation into regions only persisted for about 2 hr. In none of the ovarian or unfertilized hamster eggs is there any suggestion of the large yolk mass illustrated and described by Lewis & Wright in the mouse.

In the ovarian egg of the hamster Daleq (1954) found large mitochondrial granules in a juxta-nuclear position, during the growth of the oocyte they became more numerous and scattered in the cytoplasm. A fibrillar condensation of the cytoplasm similar to that described by us has been observed in the hamster, rat and, to a lesser extent, in the mouse, by Daleq. The fibrils persist during the first cleavage, after which they disappear. Daleq is of the opinion that the arrangement of the fibrils is of no importance in morphogenesis and only suggest a concentration of protein in the egg. The many investigations he has made (using special techniques) summarized in a recent paper (Daleq, 1952) have thrown much light on the nature of what is occurring in the cytoplasm in relationship to the changes taking place in the nucleus during cleavage. In the fertilized egg of the mouse at the pronuclear stage stained with pyronine in Tyrode's solution, the same author (1954) finds a perinuclear metachromatic staining zone comparable to the zone around the centrally placed pronuclei in the hamster.

Cell differentiation

In 1875 van Beneden first suggested that the first two blastomeres of the two-cell stage in mammalian eggs had different fates, one giving rise to ectoderm, the other to endoderm; later, Hubrecht (1902) expressed the opinion that one cell gave rise to trophoblast, the other to embryoblast. Many investigators have since studied the mammalian egg, and up to the present time no dogmatic statement can be made on the possible fate of the first two blastomeres. The problem of the

early dissociation of cells during cleavage has been discussed by Amoroso *et al.* (1942), and so it is unnecessary to consider the matter again at length. In the hamster egg the blastomeres at the two-cell stage are similar in appearance and approximately of the same size. The first evidence of histological differentiation in the egg of this mammal is not found until the morula or early blastocyst stages. The outer cells (presumably future trophoblast only) stain more darkly than the inner cell mass. Whether or not further cells, at a later stage, can be added to the inner cell mass from the outer, or vice versa, it has not been possible to determine. It has been suggested by many investigators that the staining differences between the trophoblast and the inner cell mass is due to environmental influence exerted on the egg when it reaches the uterine cavity. The differentiation of cells occurs at the time the blastocyst cavity makes its appearance, and it seems reasonable to assume, as did Heuser & Streeter (1929), that the appearance of the cavity is due to the activity of cells which have differentiated to form trophoblast. It would therefore appear more probable that the forces responsible for the segregation of cells are intrinsic and genetic rather than purely environmental.

We found no evidence, as did Heuser & Streeter in the pig, for the more precocious onset of cleavage in one of the first two blastomeres than in the other. From the two-cell stage onwards they recognized cells which show 'more active cleavage' and those which are 'more deliberate', the more actively dividing cells being the precursors of the trophoblast, the more lethargic giving rise to the embryo. Again, at no time during cleavage in the hamster egg are stages found comparable to those illustrated by Heuser & Streeter in fig. 6 or by Amoroso *et al.* in text-fig. 4 in the goat. When histological differentiation becomes apparent in the hamster egg there is a complete outer layer of darkly stained cells.

SUMMARY

A description is given of the cleavage stages of the egg of the golden hamster (*Cricetus auratus*). Details are given of techniques employed to obtain and section these eggs.

Ovulation occurs 4 hr. after copulation. The one-cell stage lasts 20 hr., the two-cell stage 25 hr., the four-cell stage 12 hr., and the eight-cell stage 8 hr. The blastocyst may be formed at the eight-cell stage.

In the living unsegmented egg and in the early stages of cleavage the cytoplasm of the egg is subdivided into a peripheral region pale in colour and a darker perinuclear zone. In the fixed egg the outer zone is coarsely granular whilst the inner zone is composed of fine granules.

During cleavage there is a marked diminution in the volume of the cytoplasmic mass.

Histological differentiation is not apparent in the blastomeres until the morula or early blastocyst stage.

Brief comments and comparisons with other mammalian eggs are made.

REFERENCES

- AMOROSO, E. C., GRIFFITHS, W. B. & HAMILTON, W. J. (1942). The early development of the goat (*Capra hircus*). *J. Anat., Lond.*, **76**, 377-406.
- BENEDEN, E. VAN (1875). La maturation de l'œuf, la fécondation et les premières phases de développement embryonnaire de Mammifères, d'après des recherches faites chez le Lapin. *Bull. Acad. Belg. Cl. Sci.* **40**, 686-736.
- BOND, C. R. (1945). The golden hamster (*Cricetus auratus*) care, breeding, and growth. *Physiol. Zool.* **18**, 52-59.
- BRUCE, H. M. & HINDLE, E. (1934). The golden hamster *Cricetus* (*Mesocricetus*) *auratus* Waterhouse. Notes on its breeding and growth. *Proc. Zool. Soc. Lond.* **104**, 361-366.
- DALCQ, A. (1952). La coloration vitale au bleu de toluidine et sa manifestation metachromatique. *C.R. Soc. Biol., Paris*, **146**, 1408-1411.
- DALCQ, A. (1954). Nouvelles données structurales et cytochimiques sur l'œuf des mammifères. *Rev. gén. Sci. pur. appl.* **61**, 19-41.
- DEANESLY, R. (1938). The reproductive cycle of the golden hamster. *Proc. Zool. Soc. Lond.* **198**, 31-39.
- GILCHRIST, F. & PINCUS, G. (1932). Living rat eggs. *Anat. Rec.* **54**, 275-287.
- GRAVES, A. P. (1945). Development of the golden hamster, *Cricetus auratus* Waterhouse, during the first 9 days. *Amer. J. Anat.* **77**, 219-251.
- HAMILTON, W. J. & LAING, J. A. (1946). Development of the egg of the cow up to the stage of blastocyst formation. *J. Anat., Lond.*, **80**, 194-204.
- HARTMAN, C. G. (1929). How large is the mammalian egg? A review. *Quart. Rev. Biol.* **4**, 373-388.
- HEUSER, C. H. & STREETER, G. L. (1929). Early stages in the development of pig embryos, from the period of initial cleavage to the time of the appearance of the limb buds. *Contr. Embryol. Carneg. Instn.* **20**, 1-30.
- HUBER, G. C. (1915). The development of the albino rat (*Mus norvegicus albinus*). *J. Morph.* **26**, 247-387.
- HUBRECHT, A. A. W. (1902). Furchung und Keimblattbildung bei *Tarsius spectrum*. *Verh. Akad. Wet. Amst.* **8**, 499-562.
- KENT, G. C., Jr. & SMITH, R. A. (1945). A study of the estrous cycle in the golden hamster, *Cricetus* (*Mesocricetus*) *auratus* Waterhouse. *Anat. Rec.* **92**, 263-272.
- KUPPERMAN, H., GREENBLATT, R. & HAIR, L. (1944). The sexual cycle and reproduction in the golden hamster (*Cricetus auratus*). (Abstract.) *Anat. Rec.* **88**, 441.
- LEWIS, W. H. & GREGORY, P. W. (1929). Cinematographs of living developing rabbit eggs. *Science*, **69**, 226-229.
- LEWIS, W. H. & HARTMAN, C. G. (1933). Early cleavage stages of the egg of the monkey (*Macacus rhesus*). *Contr. Embryol. Carneg. Instn.* **24**, 187-201.
- LEWIS, W. H. & WRIGHT, E. S. (1935). On the early development of the mouse egg. *Contr. Embryol. Carneg. Instn.* **25**, 113-143.
- MACDONALD, E. & LONG, A. J. A. (1934). Some features of cleavage in the living egg of the rat. *Amer. J. Anat.* **55**, 343-361.
- PECZENIK, O. (1942). Actions of sex hormones on oestrous cycle and reproduction of the golden hamster. *J. Endocrin.* **3**, 157-167.
- REED, C. A. & REED, R. (1946). The copulatory behaviour of the golden hamster. *J. Comp. Psychol.* **39**, 7-12.
- SAMUEL, D. M. (1944). The use of an agar gel in the sectioning of mammalian eggs. *J. Anat., Lond.*, **78**, 173-175.
- SAMUEL, D. M. & HAMILTON, W. J. (1942). Living eggs of the golden hamster (*Cricetus auratus*). *J. Anat., Lond.*, **76**, 204-208.
- SOBOTTA, J. (1924). Beiträge zur Furchung des Eies der Säugetiere mit besondere Berücksichtigung der Frage der Determination der Furchung: 1. Die Furchung des Eies der Maus (*Mus musculus*). *Z. ges. Anat.* **1**. *Z. Anat. EntwGesch.* **72**, 94-116.
- SQUIER, R. R. (1932). The living egg and early stages in its development in the guinea-pig. *Contr. Embryol. Carneg. Instn.* **23**, 223-250.
- VENABLE, J. H. (1946a). Pre-implantation stages in the golden hamster (*Cricetus auratus*). *Anat. Rec.* **94**, 105-120.

- VENABLE, J. H. (1946b). Volume changes in the early development of the golden hamster. *Anat. Rec.* **94**, 129-138.
- WARD, M. C. (1946). A study of the estrous cycle and the breeding of the golden hamster, *Cricetus auratus*. *Anat. Rec.* **94**, 139-162.
- WARD, M. C. (1948). The early development and implantation of the golden hamster, *Cricetus auratus*, and the associated endometrial changes. *Amer. J. Anat.* **82**, 231-276.

EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Photomicrograph of a living egg removed from a large follicle. Some of the corona radiata cells have been removed from the oocyte. $\times c.$ 480.
- Fig. 2. Photomicrograph of a section of an ovarian egg at $4\frac{3}{4}$ hr. after copulation. The second polar spindle is shown. $\times c.$ 640.
- Fig. 3. Photomicrograph of a section of a tubal egg 4 hr. after copulation. The sperm head and tail are seen in the cytoplasm. $\times c.$ 1400.
- Fig. 4. Photomicrograph of a section of a tubal egg 4 hr. after copulation. The second polar spindle is shown. $\times c.$ 640.
- Fig. 5. Photomicrograph of a living egg $12\frac{3}{4}$ hr. after copulation showing the early formation of the pronuclei. The remains of the sperm tail are seen. $\times c.$ 480.
- Fig. 6. Photomicrograph of a living egg $12\frac{3}{4}$ hr. after copulation. The coverglass was pressed against the egg so that it became slightly flattened. $\times c.$ 480.

PLATE 2

- Fig. 7. Photomicrograph of a living egg showing centrally placed pronuclei $23\frac{1}{4}$ hr. after copulation. The cytoplasm is composed of a darker perinuclear zone and a lighter peripheral zone. $\times c.$ 480.
- Fig. 8. Photomicrograph of a living egg showing centrally placed pronuclei 22 hr. after copulation. $\times c.$ 480.
- Fig. 9. Photomicrograph (with oil-immersion lens) of a living egg showing the finely granular cytoplasm around the pronuclei 22 hr. after copulation. $\times c.$ 480.
- Fig. 10. Photomicrograph of a section of an egg showing early pronuclei at 9 hr. after copulation. $\times c.$ 640.
- Fig. 11. Photomicrograph of a section of an egg showing almost centrally placed pronuclei $24\frac{1}{2}$ hr. after copulation. $\times c.$ 640.
- Fig. 12. Photomicrograph of a section of an egg showing eccentrically placed pronuclei $12\frac{3}{4}$ hr. after copulation. $\times c.$ 640.
- Fig. 13. Photomicrograph of a section of an egg showing three pronuclei $14\frac{1}{4}$ hr. after copulation. $\times c.$ 640.
- Fig. 14. Photomicrograph of a section of an egg showing centrally placed pronuclei 23 hr. after copulation. The cytoplasm is divisible into a coarsely granular peripheral zone and a finely granular perinuclear zone. $\times c.$ 640.

PLATE 3

- Fig. 15. Photomicrograph of a section through an egg with centrally placed pronuclei $23\frac{3}{4}$ hr. after copulation. The chromosomes are being formed. Two zones are seen in the cytoplasm. $\times c.$ 640.
- Fig. 16. Photomicrograph of a living egg $24\frac{1}{4}$ hr. after copulation. The cytoplasm in the centre of the egg has assumed an oval outline. $\times c.$ 480.
- Fig. 17. Photomicrograph of a fixed whole mount of an egg in agar $24\frac{1}{2}$ hr. after copulation. The first cleavage spindle is at the telophase. $\times c.$ 650.
- Fig. 18. Photomicrograph of a fixed whole mount of an egg in agar at the late telophase $24\frac{1}{2}$ hr. after copulation. $\times c.$ 650.
- Fig. 19. Photomicrograph of a section of an egg at the metaphase $24\frac{1}{2}$ hr. after copulation. $\times c.$ 640.
- Fig. 20. Photomicrograph of a section of an egg at the late metaphase $24\frac{1}{2}$ hr. after copulation. The cytoplasm is clearly separated into two components. $\times c.$ 640.
- Fig. 21. Photomicrograph of a section of the egg shown in fig. 17. $\times c.$ 640.
- Fig. 22. Photomicrograph of a section of the egg shown in fig. 18. $\times c.$ 640.

PLATE 4

- Fig. 23. Photomicrograph of a living two-cell stage $25\frac{1}{2}$ hr. after copulation. $\times c. 480$
 Fig. 24. Photomicrograph of a living two-cell stage 40 hr. after copulation. $\times c. 480$.
 Fig. 25. Photomicrograph of a living two-cell stage 28 hr. after copulation showing marked irregularity in the size of the blastomeres. $\times c. 480$.
 Fig. 26. Photomicrograph of a section of a two-cell stage $24\frac{1}{2}$ hr. after copulation. Finely granular cytoplasm surrounds the nucleus in each blastomere. $\times c. 640$.
 Fig. 27. Photomicrograph of a section of a two-cell stage 25 hr. after copulation. $\times c. 640$.
 Fig. 28. Photomicrograph of a section of a two-cell stage 49 hr. after copulation. $\times c. 640$.
 Fig. 29. Photomicrograph of a section of a two-cell stage $24\frac{1}{2}$ hr. after copulation. $\times c. 640$.
 Fig. 30. Photomicrograph of a living four-cell stage showing the cross arrangement of the blastomeres 64 hr. after copulation. $\times c. 480$.

PLATE 5

- Fig. 31. Photomicrograph of a living four-cell stage $60\frac{1}{2}$ hr. after copulation showing the blastomeres arranged in the same plane. $\times c. 480$.
 Fig. 32. Photomicrograph of a section of an egg at the three-cell stage $49\frac{1}{2}$ hr. after copulation. $\times c. 640$.
 Fig. 33. Photomicrograph of a section of an egg at the four-cell stage 60 hr. after copulation showing the blastomeres lying in the same plane. $\times c. 650$.
 Fig. 34. Photomicrograph of a living egg at the five-cell stage 60 hr. after copulation. $\times c. 480$.
 Fig. 35. Photomicrograph of a living egg at the six-cell stage $60\frac{1}{2}$ hr. after copulation. $\times c. 480$.
 Fig. 36. Photomicrograph of the same egg as in fig. 35 at a different focus. $\times c. 480$.
 Fig. 37. Photomicrograph of a living egg at the eight-cell stage 62 hr. after copulation. $\times c. 480$.

PLATE 6

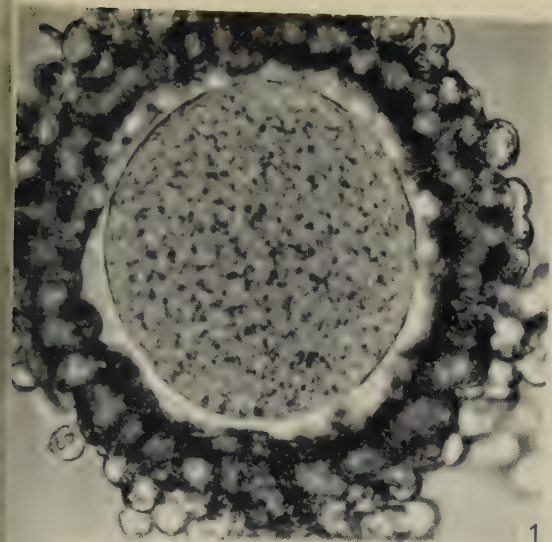
- Fig. 38. Photomicrograph of an egg at the early morula stage 71 hr. after copulation. $\times c. 480$.
 Fig. 39. Photomicrograph of a whole mount of an egg in agar at the eight-cell stage 71 hr. after copulation. $\times c. 650$.
 Figs. 40-43. Photomicrographs of the 2nd, 3rd, 4th and 7th sections of an egg at the eight-cell stage 71 hr. after copulation. Note the peripheral position of the nuclei in the cells. $\times c. 640$.
 Fig. 44. Photomicrograph of an early living blastocyst $78\frac{1}{2}$ hr. after copulation. The peripheral cells show flattening. $\times c. 480$.
 Fig. 45. Photomicrograph of an early living blastocyst 72 hr. after copulation. $\times c. 480$.

PLATE 7

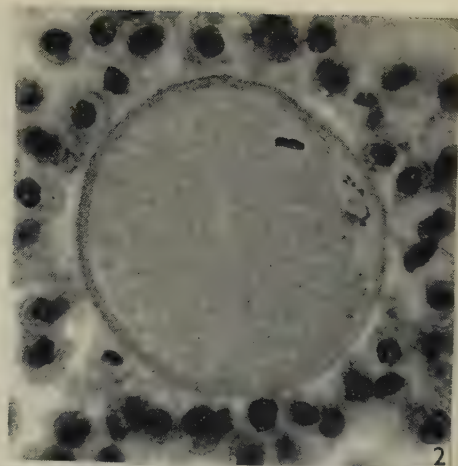
- Fig. 46. Photomicrograph of a living morula 71 hr. after copulation; the peripheral cells have become flattened. Several cells are lying outside the main mass. $\times c. 480$.
 Fig. 47. Photomicrograph of a living early blastocyst $85\frac{1}{2}$ hr. after copulation. $\times c. 480$.
 Fig. 48. Photomicrograph of a living blastocyst $85\frac{1}{2}$ hr. after copulation. $\times c. 480$.
 Fig. 49. Photomicrograph of a living blastocyst $88\frac{1}{2}$ hr. after copulation. $\times c. 480$.
 Figs. 50-54. Photomicrographs of five consecutive sections of an early blastocyst $88\frac{1}{2}$ hr. after copulation. The peripheral cells stain more darkly than the inner cells. $\times c. 640$.

PLATE 8

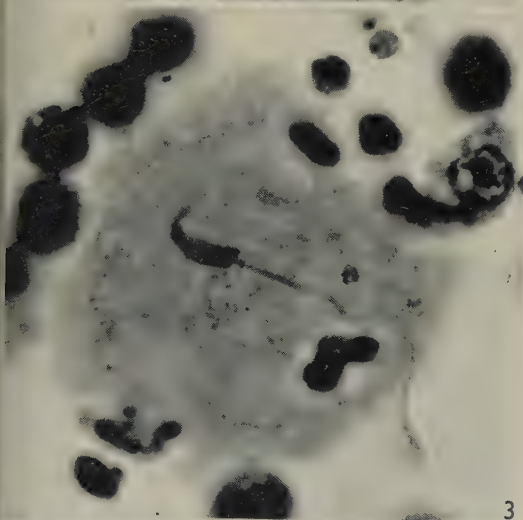
- Figs. 55-60. Photomicrographs of six consecutive sections of the blastocyst illustrated in fig. 48, $88\frac{1}{2}$ hr. after copulation. $\times c. 640$.
 Fig. 61. Photomicrograph of a section of a blastocyst in the uterus $100\frac{1}{2}$ hr. after copulation. The abembryonic pole of the blastocyst is attaching itself to the epithelium. $\times c. 640$.
 Fig. 62. Photomicrograph of a section of a blastocyst in the uterus 109 hr. after copulation. The trophoblast cells of the abembryonic pole of the blastocyst have penetrated between the epithelial cells. A few endodermal cells have differentiated from the inner cell mass. $\times c. 640$.



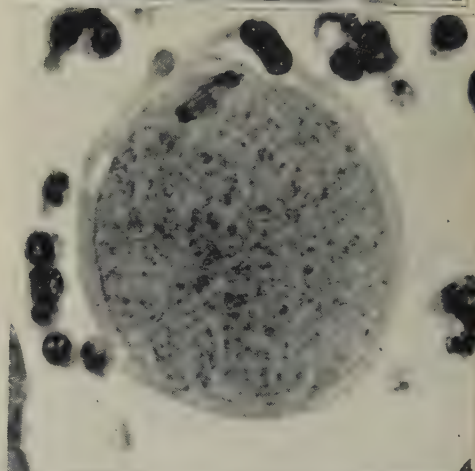
1



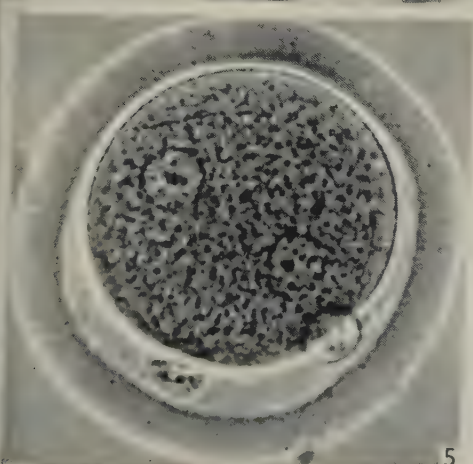
2



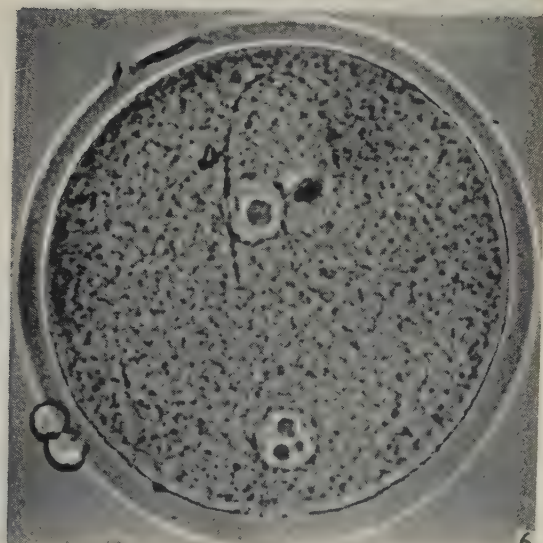
3



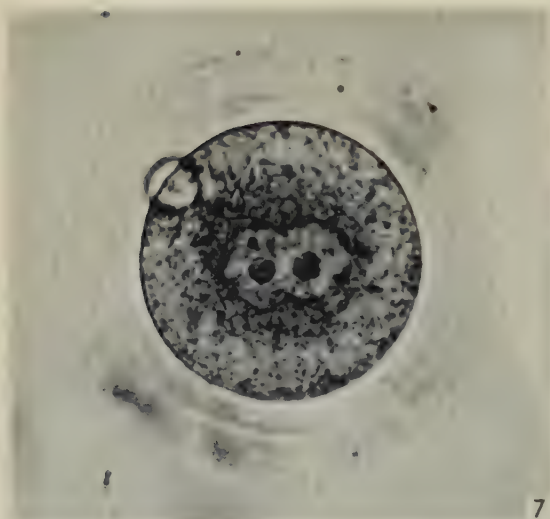
4



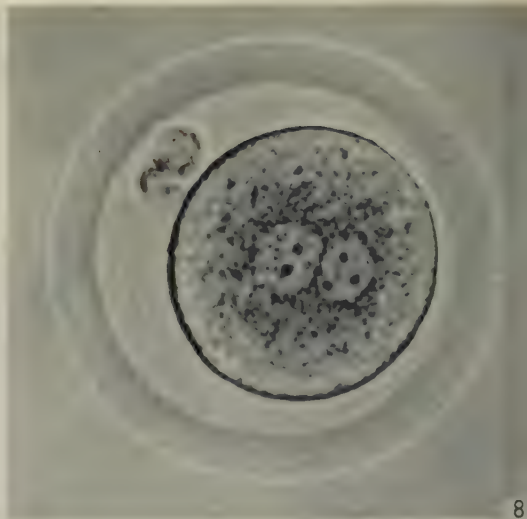
5



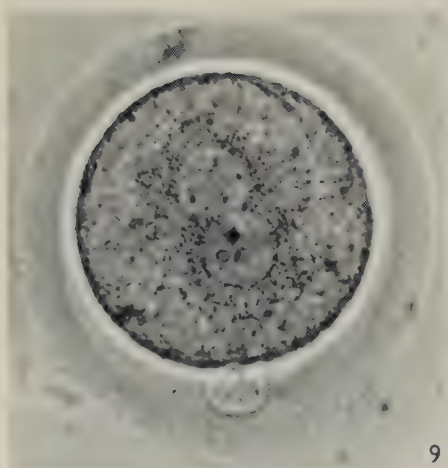
6



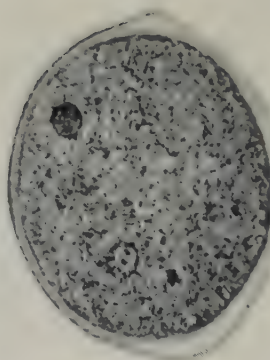
7



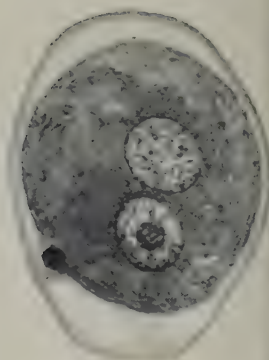
8



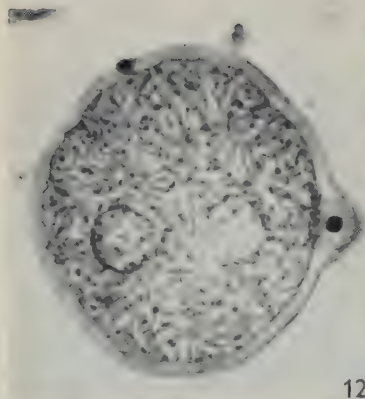
9



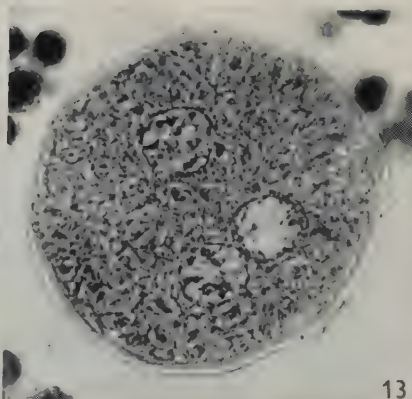
10



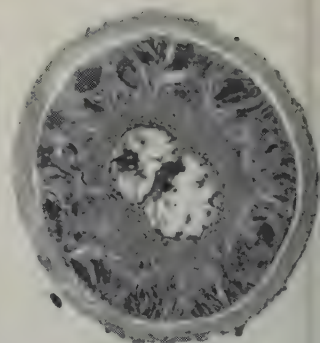
11



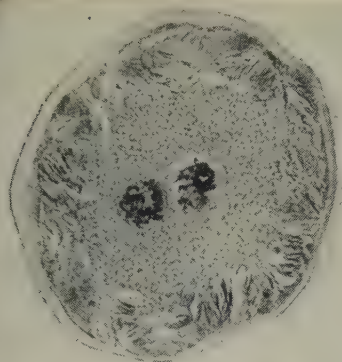
12



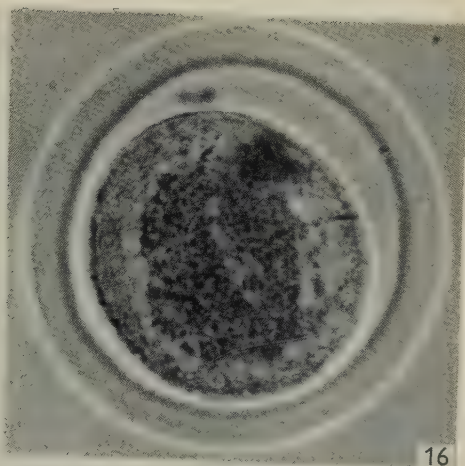
13



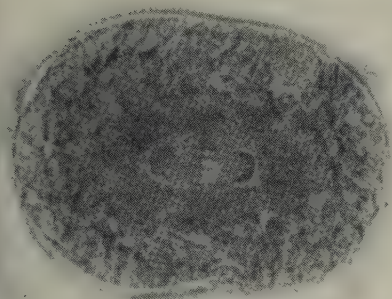
14



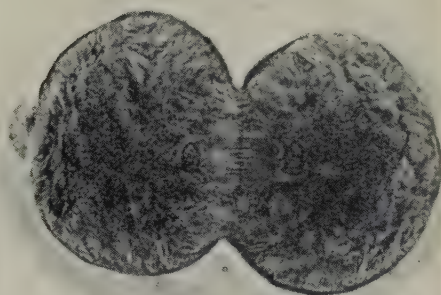
15



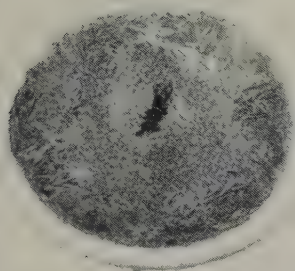
16



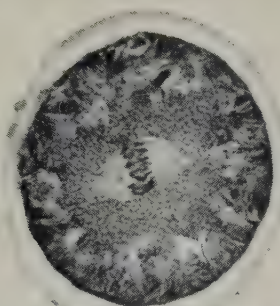
17



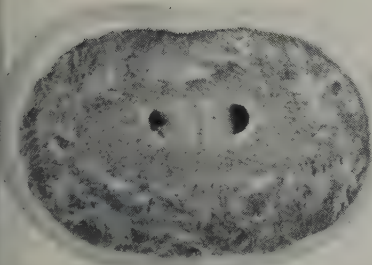
18



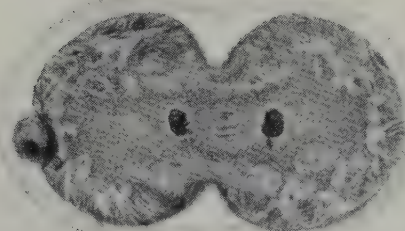
19



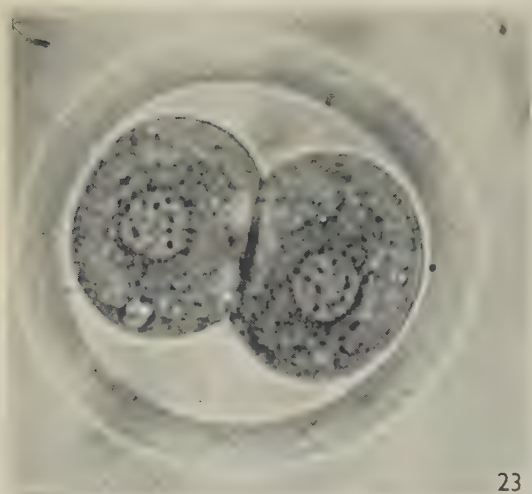
20



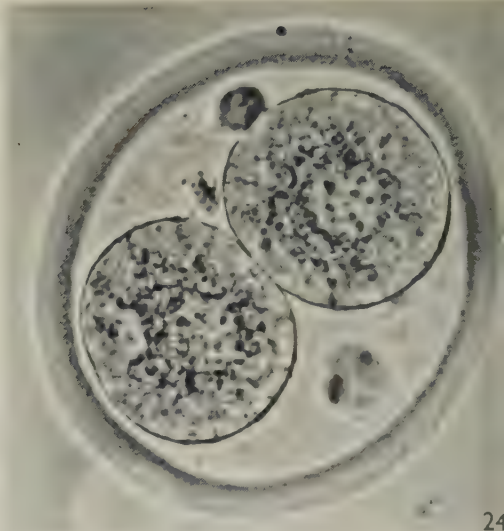
21



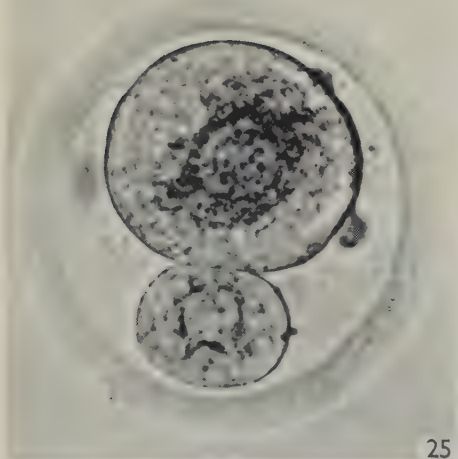
22



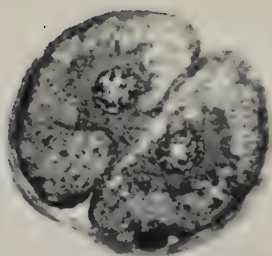
23



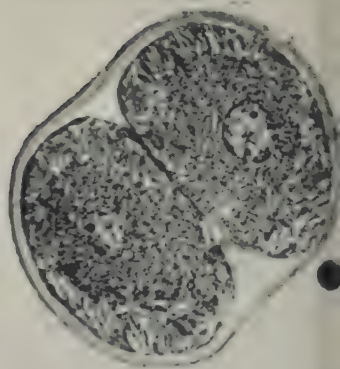
24



25



26



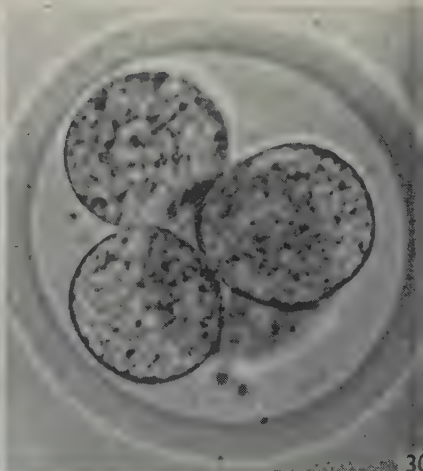
27



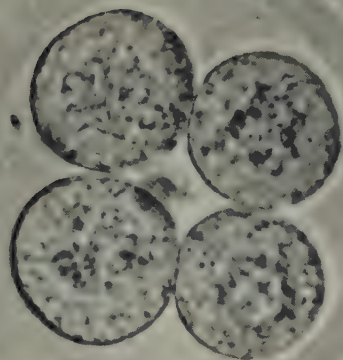
28



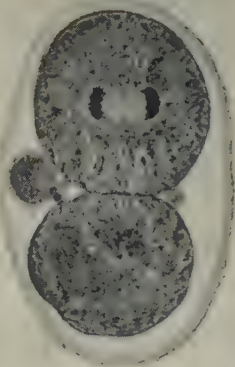
29



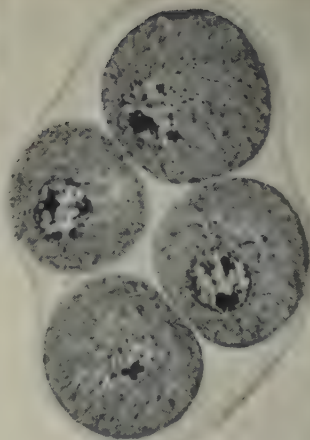
30



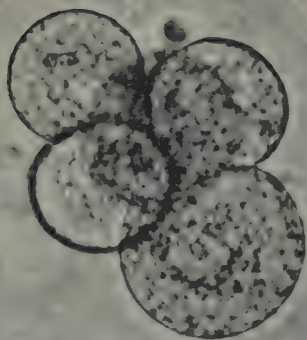
31



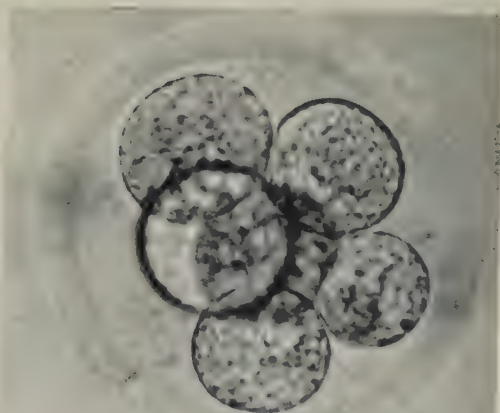
32



33



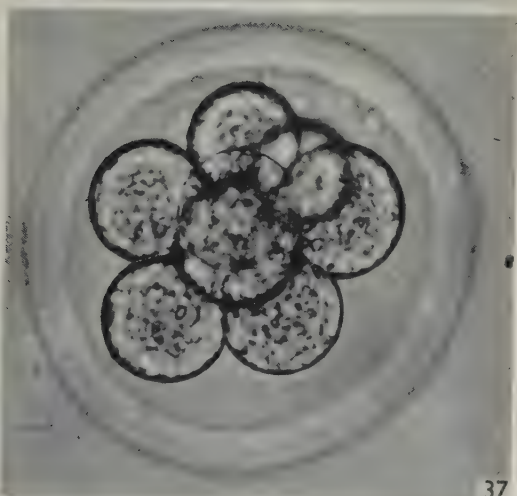
34



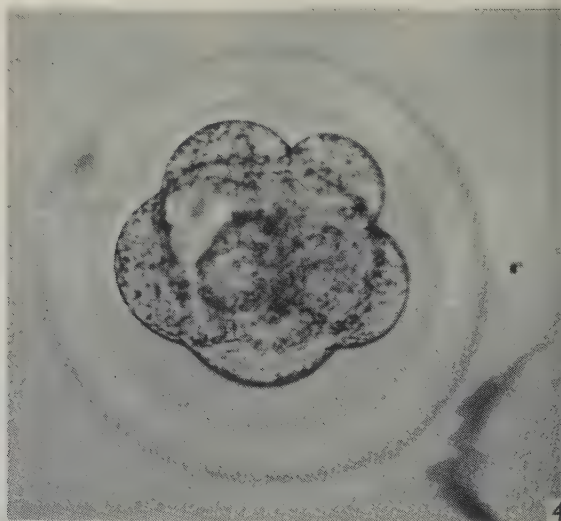
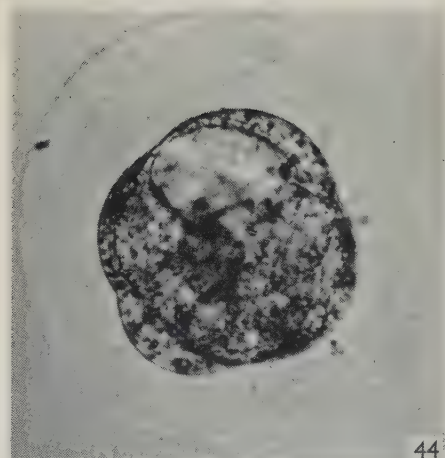
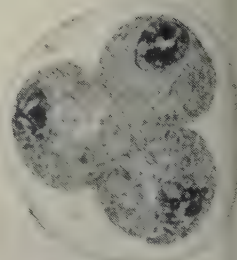
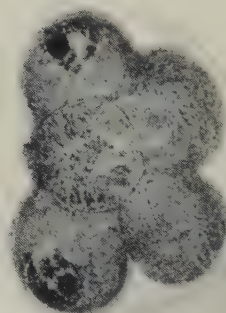
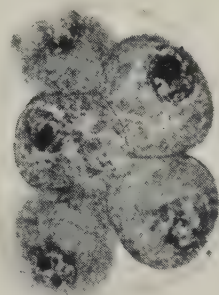
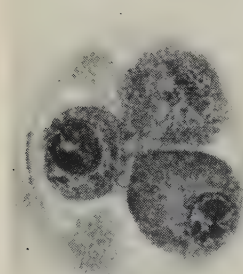
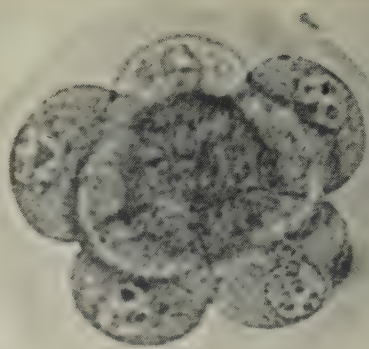
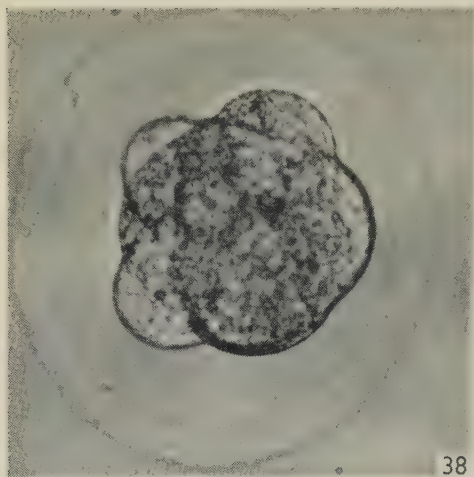
35

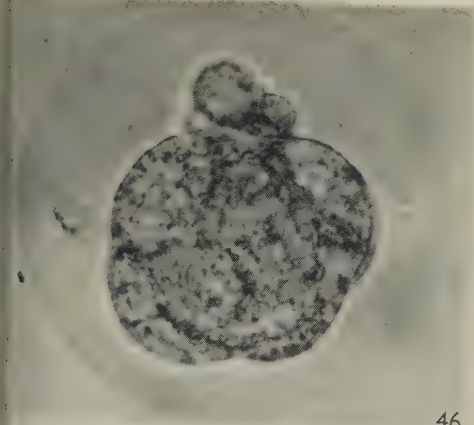


36

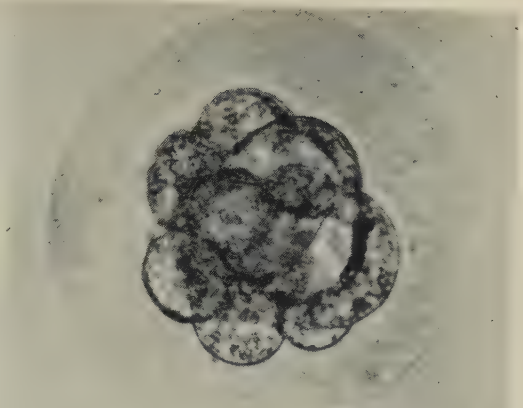


37

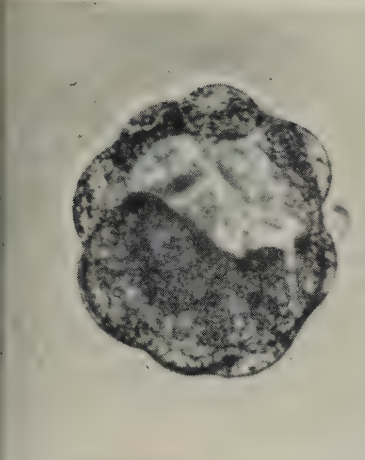




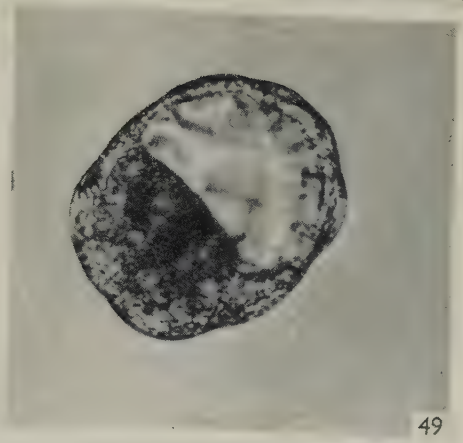
46



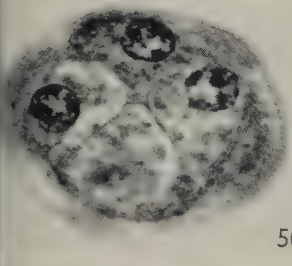
47



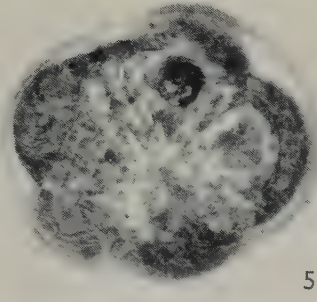
48



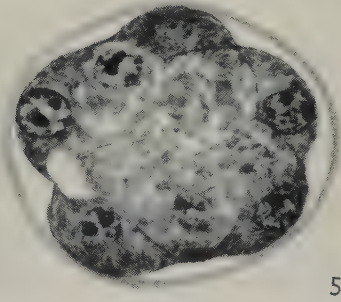
49



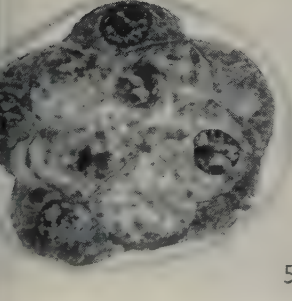
50



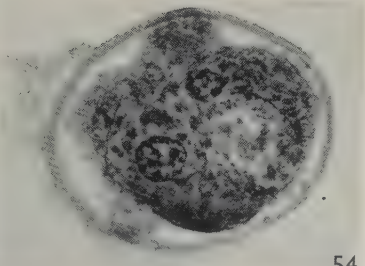
51



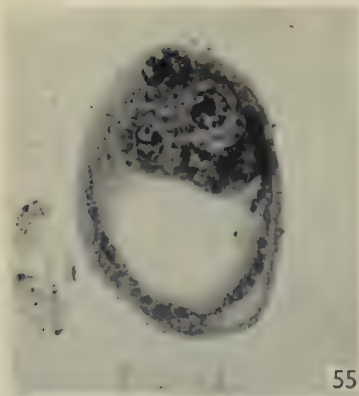
52



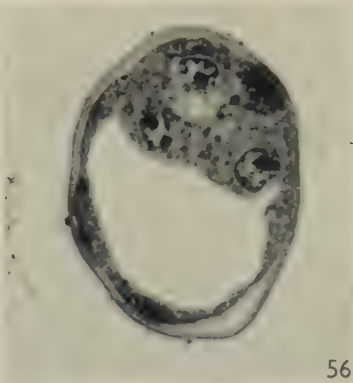
53



54



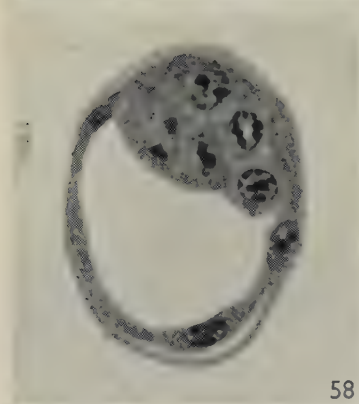
55



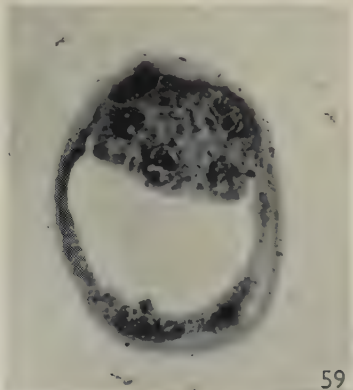
56



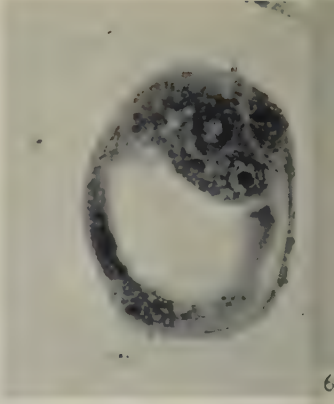
57



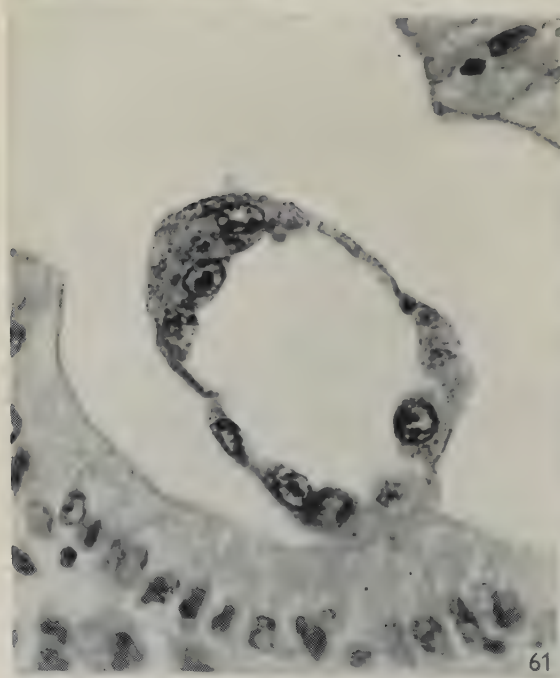
58



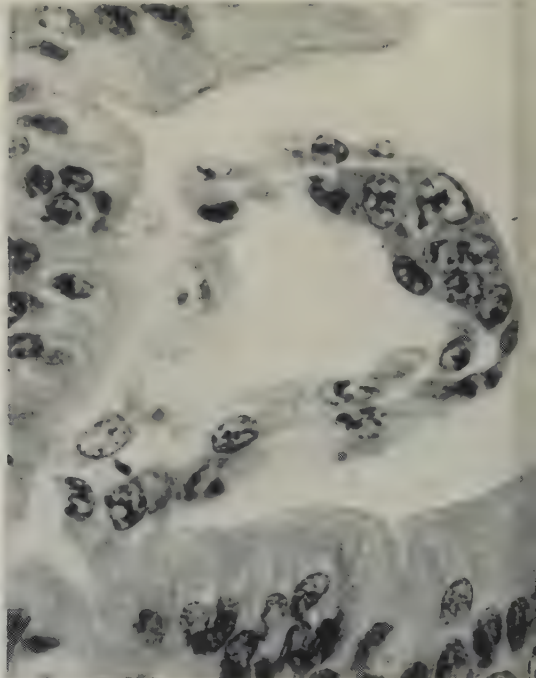
59



60



61



THE EFFECT OF CORTISONE ACETATE ON THE RESPONSE OF THE REGIONAL LYMPH NODE TO A SKIN HOMOGRAFT

By R. J. SCOTHORNE

Department of Anatomy, University of Glasgow

INTRODUCTION

In a previous paper (Scothorne & McGregor, 1955) the changes which occur in the lymph nodes and spleen following skin grafting in the rabbit were described, and the main conclusion was that a skin homograft provokes in the first regional lymph node a specific cellular response. After homografting, numerous 'large lymphoid cells' appear, principally within the enlarged cortex of the node. These cells are 14–18 μ in diameter, and have large nuclei with one or more prominent nucleoli, and abundant pyroninophilic (basophilic) cytoplasm. This large lymphoid cell response is specific in the sense that it is not provoked by autografts of skin. It is fully developed within 4 days of homografting, persists until the time of graft breakdown on about the 6th or 7th day, and thereafter subsides fairly rapidly. Arguments were advanced that the large lymphoid cell response is not merely a specific homograft reaction but that it is in fact the source of the antibodies which are believed to be responsible for homograft destruction.

Billingham, Krohn & Medawar (1951*a, b*) have shown that the survival of skin homografts in the rabbit may be considerably prolonged by the systemic administration of 10 mg. of cortisone acetate daily, and also by the local application of cortisone acetate to the graft itself, in very much smaller dosage. The aim of the present investigation was to determine the effect of cortisone on the development of the large lymphoid cell response. If this response is the source of the systemic immunity, one would expect it to be delayed or suppressed by the use of cortisone.

MATERIAL AND METHODS

The experimental procedure was similar to that followed previously (Scothorne & McGregor, 1955). Young adult rabbits of both sexes, weighing between 2 and 2.5 kg. were used. A single full thickness graft of skin (30 \times 15 mm.) was cut from the dorsum of the ear and exchanged with a similar graft from another rabbit. Unrelated rabbits, usually of different breeds, were used in each case. The grafts were fixed by interrupted silk sutures, and no dressings were used.

The effect of cortisone on the development of the large lymphoid cell response was examined in two separate experiments. In Exp. I, the rabbits were injected beneath the skin of the lower back with 1 ml. of a suspension containing 10 mg./ml. of cortisone acetate. Injections were given daily from the day of operation until the time of killing at 4, 5, 6, 12 and 16 days after operation.

In Exp. II, one member of each pair of rabbits received a local application to the surface of the graft of 0.1 ml. of a suspension containing 20 mg./ml. of cortisone

acetate, while the other member of each pair was injected with a similar dose of cortisone subcutaneously in the ear proximal to the graft. Cortisone was given on the day of operation (day 0) and on days 3 and 6 after grafting and the animals were killed on the 6th to 8th day.

The graft was fixed in Bouin's fluid, embedded in paraffin, sectioned and stained with haematoxylin and eosin. The first regional node draining the ear on both the operated and unoperated (control) sides was removed, weighed, fixed in 4 % neutral formaldehyde, sectioned at 8μ and stained with methyl-green-pyronin. The method of staining was that described by Pearse (1953). Pyronin G (B.D.H.) proved the most satisfactory of a number of samples tested. The spleen was also removed and weighed.

RESULTS

Experiment I (daily systemic administration of 10 mg. cortisone acetate)

A. The graft

In untreated control animals, autografts and homografts behave similarly during the first 4 or 5 days. For the first 2 days the graft is pale and translucent, and its surface is flush with, or slightly below, that of the surrounding host skin, which is somewhat hyperaemic. By the 4th day the graft is securely healed in position. It is opaque, level with, or slightly raised above, the surrounding skin, and, since it is now fully revascularized (Scothorne & McGregor, 1953), pink or red in colour. Microscopic sections of both autografts and homografts at this stage show two important features—a great increase in the thickness of the epidermis, due to cellular hyperplasia and hypertrophy, and the invasion of the base of the graft by host granulation tissue (cf. Medawar, 1944).

During the following week or more, skin autografts gradually assume a normal appearance and are eventually scarcely distinguishable from the surrounding skin.

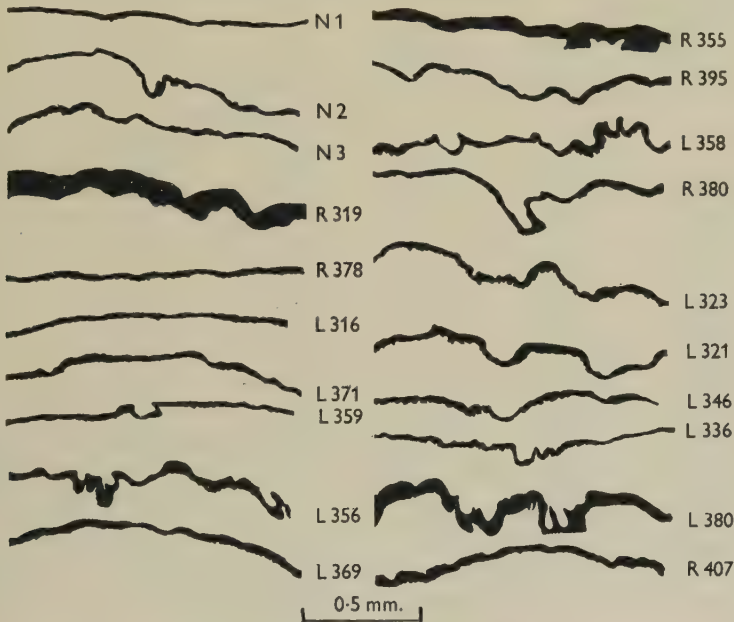
After the initial healing period, homografts behave differently, and by about the 6th or 7th day the external signs of graft destruction are apparent. There is a characteristic change in the colour of the graft, from a healthy pink to dusky red or slate-grey. The graft becomes swollen and raised above the surrounding host skin, dark red, black, or sometimes greenish-yellow, hard and dry, and eventually it separates as a scab.

The absence of any dressing enables one to make repeated examinations of the graft, and I have found the change in colour to be quite a sensitive indicator of the time of onset of graft destruction. Histological sections of grafts removed at the time of darkening in colour invariably show the microscopic signs of epidermal destruction—nuclear pyknosis, karyorrhexis and karyolysis, acidophilia of the cytoplasm in the stratum germinativum, and the separation, in localized areas, of epidermis and dermis. The graft is infiltrated by large numbers of 'small round cells', principally lymphocytes.

With homografts of the size and type used in these experiments, the *onset* of graft destruction may occur as early as the 5th and as late as the 8th day after grafting, but in the great majority of cases it is found on the 6th or 7th day.

As was shown by Billingham *et al.* (1951*a*), the systemic administration of 10 mg. cortisone daily prolongs the survival of skin homografts three- or four-fold, the

longest period of survival in their experiments being over 40 days, the shortest, over 20. In the present experiment, homografts were studied up to the 16th day, and as expected, they remained healthy throughout the experimental period. At the 16th day their appearance differed little from that at the time of transplantation. They were pale, unthickened and translucent. Healing of the graft to its bed was obviously impaired, so that at autopsy it could be stripped away from its bed by means of forceps, whereas in untreated animals the graft is firmly united with the graft bed and must be freed with a scalpel.



Text-fig. 1. Camera lucida profiles of epidermis, to illustrate epidermal thickness. N1, N2 and N3: normal controls. R319: 4-day homograft, untreated. R378, L316, L371 and L359: 4-day homografts, systemic cortisone. L356, L369: 5-day homografts, systemic cortisone. R355: 6-day homograft, untreated. R395 and L358: 6-day homografts, systemic cortisone. R380 and L323: 12-day homografts, systemic cortisone. L321, L346 and L336: 16-day homografts, systemic cortisone. L380: 7-day homograft. Cortisone injected subcutaneously between graft and regional node; total dosage, 6 mg. R407: 7-day homograft. Cortisone applied locally to graft; total dosage, 6 mg.

After removal, grafts on cortisone-treated hosts resemble normal skin in thickness, pliability, translucency and microscopic appearance.

Text-fig. 1 shows camera lucida profiles of the epidermis of grafts on cortisone-treated animals and on various controls. Even at 16 days the epidermis of grafts on cortisone-treated hosts is little thicker than that of ungrafted skin. Billingham *et al.* (1951a) had already noted, in grafts on animals treated for 6 days with cortisone, the absence of cell proliferation in the epidermis, and they advanced good evidence for believing that the mitotic indolence of these grafts is due not to a direct inhibitory action of cortisone on the epidermis, but to inadequate vascularization of the graft, resulting from suppression of invasion of the graft by host granulation

tissue. If this idea is correct, the vascularity of grafts on cortisone-treated hosts is deficient even at 16 days, and this view is supported by the pallor and translucency of the graft and by the paucity of vessels penetrating the base of the graft from the graft bed.

B. The regional node

(1) *Weight.* Table 1 shows the weights of the regional nodes from the operated and control sides and of the spleen in homografted cortisone-treated animals and in a variety of controls.

Table 1

A. Systemic administration of cortisone, 10 mg. daily

Serial no.	Days of injection	Day of sacrifice	Wt. of node, operated side (mg.)	Wt. of node, unoperated side (mg.)	Wt. of spleen (mg.)
L 359	0-3	4	60	40	720
L 371	0-3	4	115	50	900
L 316	0-4	4	80	50	750
R 378	0-4	4	80	45	850
L 369	0-4	5	70	60	650
L 356	0-4	5	80	50	900
L 358	0-6	6	60	40	830
R 395	0-6	6	80	30	680
L 323	0-11	12	170	110	770
R 380	0-11	12	70	70	760
L 336	0-15	16	110	50	540
L 346	0-15	16	50	50	430
L 321	1-16	16	90	20	460
R 396	1-16	16	70	70	850
Mean and S.E.			84.6 ± 8	52.5 ± 5.7	720 ± 41

B. Controls, not receiving cortisone

No. of rabbits	Nature of experiment and status of graft	Wt. of node, operated side (mg.)	Wt. of node, unoperated side (mg.)	Wt. of spleen (mg.)
6	Normal controls	Left = 132 ± 10.1	Right = 127 ± 7.6	1550 ± 183
8	Autografts	198 ± 21	119 ± 14	1320 ± 94
6	Homografts, days 4-6, before breakdown	317 ± 19.9	156 ± 17.9	1740 ± 119
6	Homografts, days 5-7, after breakdown	465 ± 67	134 ± 16.9	1706 ± 250

The figures make it clear that the systemic administration of cortisone does not merely prevent the increase in weight of the ipsilateral regional node which occurs in untreated homografted animals, but actually reduces the weight below that in unoperated controls. The node on the operated side is still, however, almost invariably heavier than that on the control (unoperated) side.

The work of Krohn & Zuckerman (1954) and of Scothorne & McGregor (1955) has already indicated that the spleen is of little importance as a source of antibody production against orthotopic skin homografts, and the reduction in weight of the spleen in cortisone-treated, homografted animals is simply an indication of the well-

known lympholytic action of cortisone, and serves only as a control of the potency of the cortisone suspension. Since the spleen does not show a large lymphoid cell response to orthotopic skin grafts, it has not been studied microscopically in the present investigation.

(2) *Histological appearances.* Pl. 1, fig. 1, is a low-power view of the first regional node from a normal control animal, and Pl. 1, fig. 2, shows the appearance of the node in an untreated homografted animal 4 days after operation. The enlargement of the node is obvious. It involves principally the so-called 'tertiary cortical nodules'.

Nodes from homografted animals receiving cortisone present a very different appearance. Pl. 1, fig. 3, shows a section through the node on the 4th day, and is typical of the appearance at this stage. The node is smaller than normal, but its general topography is essentially unchanged. The great enlargement of the tertiary cortical nodules, which is such a striking feature of nodes from untreated homografted animals, is completely absent here.

In one animal at 4 days the node does show a slight enlargement of the tertiary cortical nodules (Pl. 1, fig. 4), but it is clearly very much less than that seen in untreated homografted animals (cf. Pl. 1, figs. 2 and 4).

At each of the subsequent stages examined, the node was smaller than normal and showed either a slight enlargement of the cortex (6 days, Pl. 1, fig. 5) or none (16 days, Pl. 1, fig. 6).

The most interesting microscopic feature, however, is the reduction in the number of large lymphoid cells in the majority of nodes at all stages. The paucity of these cells is so obvious that in most cases cell counts are superfluous. Since, however, in two or three cases there were regions of the node showing significant concentrations of large lymphoid cells, counts were made in each node of the series. All large lymphoid cells were counted in sixteen small squares of an eyepiece graticule in each of ten high-power fields, randomly selected within the tertiary cortical nodules, and all the small lymphocytes were counted in one small square of ten similar high-power fields. The results are given in Table 2.

Comparison of the results for the untreated homografted animals, R319 and R350, with those for the cortisone-treated group shows that in most cases the large lymphoid cell response is greatly reduced in cortisone-treated animals. In the three exceptional cases, L371, L316 and R395, the *concentration* of large lymphoid cells is not convincingly lower than that in untreated animals. If, however, allowance is made for the facts (1) that the node is some 5-6 times smaller in the cortisone-treated animals and (2) that in untreated animals the large lymphoid cells constitute a somewhat larger proportion of the total cell population, then it will be evident that the *total number* of large lymphoid cells is smaller in the nodes from cortisone-treated animals by a factor of *at least* 5 or 6.

Finally, there are no erythrocyte-filled macrophages in the medullary sinuses of nodes from homografted cortisone-treated animals. These are a constant feature of nodes from untreated homografted animals, in which their presence is attributed to drainage of extravasated erythrocytes from the graft bed to the regional node by way of lymphatics. Their absence in cortisone-treated animals may indicate some impairment in the lymphatic drainage of the graft bed, and therefore, presumably, of the graft itself.

Table 2. *Effect of cortisone on numbers of large lymphoid cells in regional node, operated side*

Serial no.	Nature of experiment	Wt. of node (mg.)	Large lymphoid cells/field (mean of 10 fields)	Small lymphoid cells/field
L384 } R356 } R349 }	Normal control	{ 110 170 110	1 1.9 5.5	62 67 68
R319	Homograft, untreated 4th day	370	63	28
R350	Homograft, untreated 6th day	410	65	32
L371 } R378 } L359 } L316 }	Homograft, cortisone treated 4th day	{ 60 80 115 80	38 4 1.5 49	39 44 59 37
L356 } L369 }	Homograft, cortisone treated 5th day	{ 80 70	5.3 3	47 56
R395 } L358 }	Homograft, cortisone treated 6th day	{ 80 60	50 14.3	45 55
R380 } L323 }	Homograft, cortisone treated 12th day	{ 70 170	4.2 20	46 45
L336 } L321 } L346 } R396 }	Homograft, cortisone treated 16th day	{ 110 90 50 70	0.3 13.2 1.5 3.1	70 57 71 66

Experiment II

This experiment was designed to compare the effect on the development of the large lymphoid cell response (i) of the local application of cortisone to the graft itself, and (ii) of the subcutaneous injection of cortisone proximal to the graft. Cortisone was administered on days 0, 3 and 6 after grafting, the total dosage in each case being 6 mg.

A. The graft

As might be expected from the results of Billingham *et al.* (1951*b*), there was a marked difference in the behaviour of grafts receiving a local application of cortisone as compared with that of grafts on animals injected with cortisone subcutaneously proximal to the graft.

In the 'injected group', the grafts behaved like those on untreated animals. By the 4th day, they were opaque, slightly thickened and, as judged by their pink colour, fully vascularized. The animals were killed at the time of onset of graft breakdown, which occurred within the usual time range of 6-8 days. The grafts were adherent to the graft bed, opaque, firm and thickened, and microscopically they were indistinguishable from breaking-down untreated grafts. The epidermis showed the usual thickening (Text-fig. 1, L 380).

Grafts receiving a local application of cortisone reacted differently, and their appearance up to the time of sacrifice was essentially similar to that of grafts in Exp. I. They remained pale, unthickened, translucent and were feebly adherent to the graft bed. Microscopically they differed little from normal skin, and the epidermis was unthickened (Text-fig. 1, R 407).

B. *The regional node*

Table 3 shows the weight of the regional node on the operated and control sides and of the spleen of the two groups of animals in Exp. II.

Table 3

Serial no.	Day of sacrifice	State of graft	Wt. of node, operated side (mg.)	Wt. of node, unoperated side (mg.)	Wt. of spleen (mg.)
A. Injection of cortisone proximal to graft					
R 414	7	Dying	520	70	1010
L 363	8	Dying	390	130	650
L 380	7	Dying	500	100	1950
L 364	6	Dying	260	70	1100
Mean and S.E.			418 \pm 60	93 \pm 15	1178 \pm 275
B. Local application of cortisone to graft					
R 387	7	Healthy	190	130	2850
L 360	8	Healthy	150	160	1200
R 407	7	Healthy	140	60	1700
Mean and S.E.			160 \pm 15	116 \pm 30	1916 \pm 488

In the 'local application' group, in which the grafts were still healthy at the time of sacrifice, the mean weight of the regional nodes is only slightly greater than that in unoperated controls. In the 'local injection' group, in which the grafts were dead at the time of sacrifice, the weight of the regional node is increased and comparable with that seen in untreated homografted animals.

Microscopic sections showed that, in the 'local application group', the node was essentially normal in size and general topography (cf. Pl. 1, figs. 1, 7) and the large lymphoid cell response was markedly suppressed, whereas in the other group, the node was considerably enlarged, similar in general appearance to nodes from untreated homografted animals (cf. Pl. 1, figs. 2, 8), and the large lymphoid cell response was fully developed. The results of counts of large lymphoid cells in the two groups of animals, carried out as in Exp. I, are shown in Table 4.

Table 4

Serial no. host	Serial no. donor	Cortisone administration	Status of graft and day of sacrifice	Mean no. large lymphoid cells/field
R 407	L 380	Local application	Healthy (7)	6.6
L 380	R 407	Injection	Dead (7)	50.6
R 387	R 414	Local application	Healthy (7)	2.6
R 414	R 387	Injection	Dead (7)	41
L 360	L 363	Local application	Healthy (8)	3.3
L 363	L 360	Injection	Dead (8)	54

In both groups, the regional lymph node of the operated side showed numerous erythrocyte-filled macrophages in the medullary sinuses, an unexpected finding in view of the results of Exp. I.

DISCUSSION

There is general agreement that the lymphatic tissues are the principal source of antibodies against known antigens, and the work of Mitchison (1954) and of Billingham, Brent & Medawar (1954) has established that they also play an important role in the development of the immunity responsible for homograft destruction. In the previous paper (Scothorne & McGregor, 1955) it was suggested that the large lymphoid cells which appear in the regional lymph node are the source of the antibodies against skin homografts. This hypothesis has been tested in two experiments designed to determine the effect of cortisone on the development of the large lymphoid cell response. In Exp. I, the systemic administration of cortisone in high dosage has been shown to prolong graft survival and to suppress or reduce the large lymphoid cell response. In Exp. II, local application of cortisone in small dosage to the surface of the graft similarly prolongs its survival and suppresses the response, whereas the injection of cortisone in the same small dosage proximal to the graft fails both to suppress the response and to prolong graft survival. The results in each case are entirely consistent with the view that the large lymphoid cell is actively concerned in the development of the immune response to skin homografts.

The experiments also throw some light on the mechanism of action of cortisone in prolonging homograft survival. In Exp. I, cortisone reaches both the graft and the node by way of the blood stream, and the prolongation of graft survival might be due to one or more of the following factors:

- (1) A primary action on the graft, reducing the capacity of the graft to *elicit* the immune response.
- (2) A primary action on the node, reducing the capacity of the node to *respond* to the antigens reaching it from the graft.
- (3) A secondary action on the graft, protecting it from the effect of antibodies reaching it from the node.

The experiments of Billingham *et al.* (1951*a, b*) definitely exclude the third possibility, and Exp. II of the present study provides an apparently crucial test between the other two. If the primary effect of cortisone were on the node, then local injection between the graft and the node should be *at least* as effective in prolonging homograft survival as local application. Indeed, since there is no reason to doubt that more cortisone will reach the node when it is injected than when it is applied locally to the graft, injection should be *more* effective than local application, if the primary action were on the node. The reverse is in fact the case: when cortisone is applied locally the grafts remain entirely healthy at the time of sacrifice, whereas they are invariably dying when cortisone is injected, in the same small dosage, between graft and node. These results seem unequivocal and suggest that cortisone exerts its effect primarily on the *graft*, reducing its ability to *elicit* the immune response.

If it is accepted that cortisone acts primarily by reducing the effective antigenicity of the graft, consideration must be given to the mechanism of this action. There are at least two possibilities:

- (1) It seems likely from the work of Billingham & Sparrow (1954) that the epidermis of a skin homograft plays a more important part than the dermis in eliciting

immunity. It is also evident that, in untreated animals, the effective total quantity of antigenic material is greatly increased by the cellular hypertrophy and hyperplasia of the epidermis occurring at the 4th to 6th days after transplantation. Not only is there an increase in the number and size of the cells, but their protein-synthetic activity is also enhanced, as evidenced by a marked increase in their cytoplasmic content of ribose nucleic acid (Scothorne & Tough, 1952; Scothorne & Scothorne, 1953). Since cortisone when injected systemically in large dosage, or applied locally in small dosage, prevents epidermal hypertrophy and hyperplasia, it must necessarily reduce the total quantity of available antigens.

(2) In those animals receiving cortisone in large systemic dosage or by local application, there is evident delay in the healing of the graft to the graft bed and in the rate of revascularization of the graft, which gives every appearance of surviving in an indolent condition without becoming intimately associated with the host. Since there is good evidence, summarized in the previous paper (Scothorne & McGregor, 1955), that the lymphatics of the graft and graft bed provide the principal route for the transport of antigenic material from graft to host, any interference with the lymphatic drainage of the graft would be expected to reduce the effective antigenicity of the graft. While no direct evidence is available about the rate of regeneration of lymphatics in skin grafts in cortisone-treated animals, Cavalli (1935) has reported that skin autografts in untreated animals are fully supplied by lymphatic vessels by the 4th to 5th days after transplantation, and this figure agrees closely with that established by Scothorne & McGregor (1953) for revascularization. It seems reasonable to suppose that the obvious delay in revascularization of grafts in cortisone-treated animals will be paralleled by a delay in lymphatic regeneration.

There is at present no direct evidence bearing on this view, and the available indirect evidence is equivocal. In Exp. I, there are no erythrocyte-filled macrophages in the medullary sinuses of the regional node, whereas they are abundant in the nodes of untreated homografted animals. This observation may indicate that large systemic doses of cortisone impair the lymphatic drainage of the *graft bed* (which, of course, is the source of the erythrocytes) and, presumably, of the graft itself. In Exp. II, on the other hand, erythrocyte-filled macrophages are abundant in the regional node in both groups of animals. It must be concluded in this case that lymphatic drainage of the *graft bed* is not interfered with, but no definite decision can be reached about the lymphatic drainage of the graft itself.

The hypothesis that cortisone acts by retarding lymphatic regeneration within the graft has been tested further by delaying cortisone administration until the 4th day after grafting, when it may be assumed that lymphatic regeneration is well advanced. If cortisone acts by preventing the passage of antigens from graft to node, then in this type of experiment graft survival should not be prolonged beyond the usual time of 6–8 days. The results of preliminary experiments have been very variable. Of six rabbits, graft destruction, confirmed histologically, was found in one at 7 days, one at 8 days and two at 11 days after grafting. In the remaining two animals the graft was essentially healthy at the 7th and 13th days respectively. These results, variable in themselves, are also at variance with those reported by Billingham *et al.* (1951*a*), who, in a similar experiment, found survival of grafts to 15–20 days in three rabbits. The reason for the inconsistency of these results is not

immediately obvious, but it seems to me that the basic design of the experiment is unsound. Cortisone administration is begun at the arbitrarily selected time of 4 days, when lymphatic regeneration, which can scarcely be expected to proceed at a uniform rate in all animals, may be extensive in some and slight in others. Moreover, cortisone may reduce the permeability to antigenic proteins of those lymphatics which have regenerated. These variables are uncontrolled and uncontrollable.

In Exp. II, by contrast, the results seem quite unequivocal, and in the absence of further direct information about the effect of cortisone on the rate of regeneration, and also on the permeability, of lymphatic vessels within the graft, the hypothesis that cortisone acts primarily by reducing the effective antigenicity of the graft seems to merit serious consideration.

SUMMARY

1. Cortisone, administered systemically in a dosage of 10 mg./day, or applied locally to the surface of the graft in a dosage of 2 mg. every third day, prolongs the survival of skin homografts and suppresses or reduces the development of the 'large lymphoid cell response' in the regional lymph node in rabbits.

2. Cortisone injected subcutaneously between the graft and the regional node in a dosage of 2 mg. every third day does not prolong homograft survival and does not reduce the 'large lymphoid cell response' in the regional node.

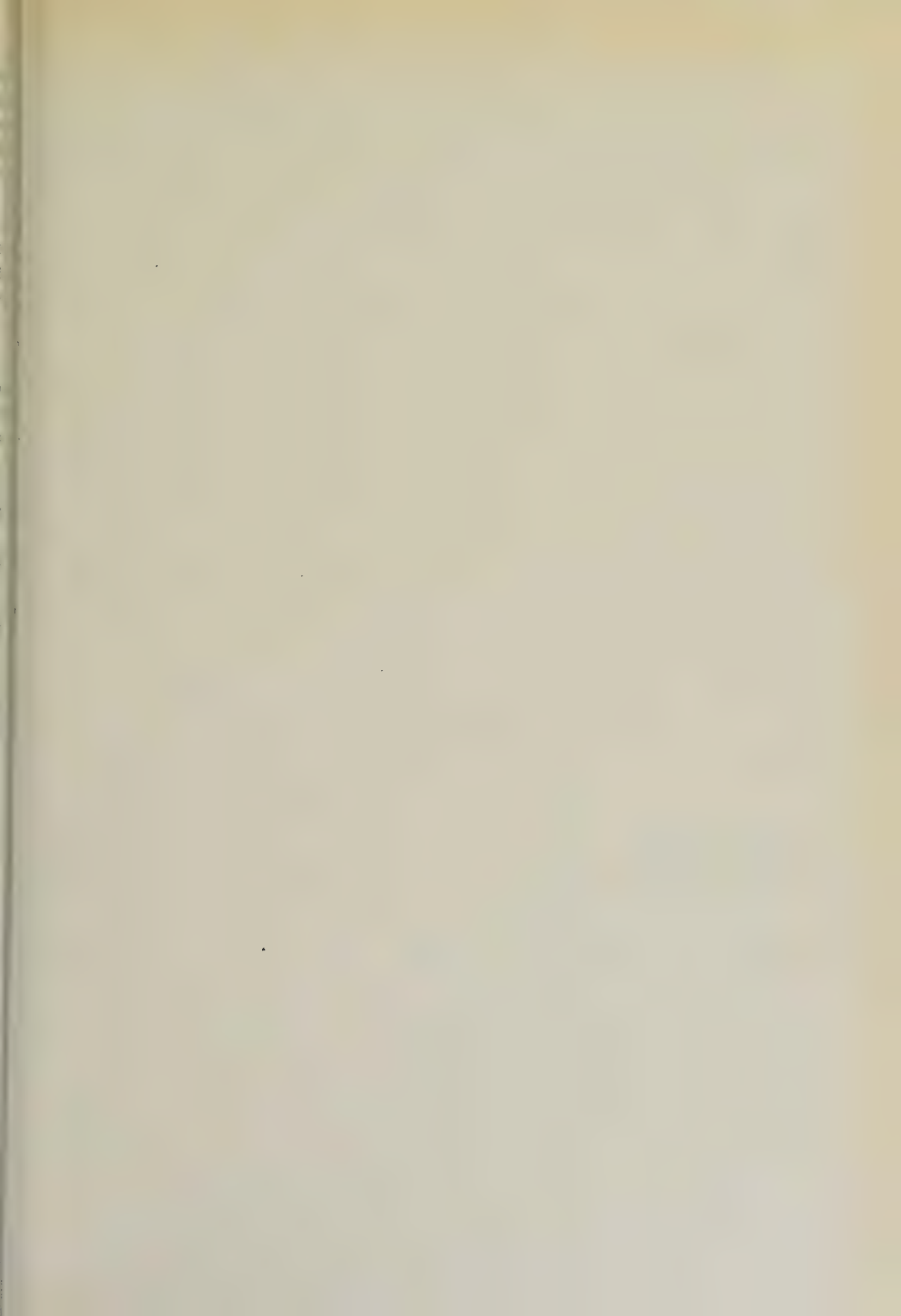
3. These findings are consistent with the hypothesis that the large lymphoid cell is actively involved in the production of antibodies against skin homografts.

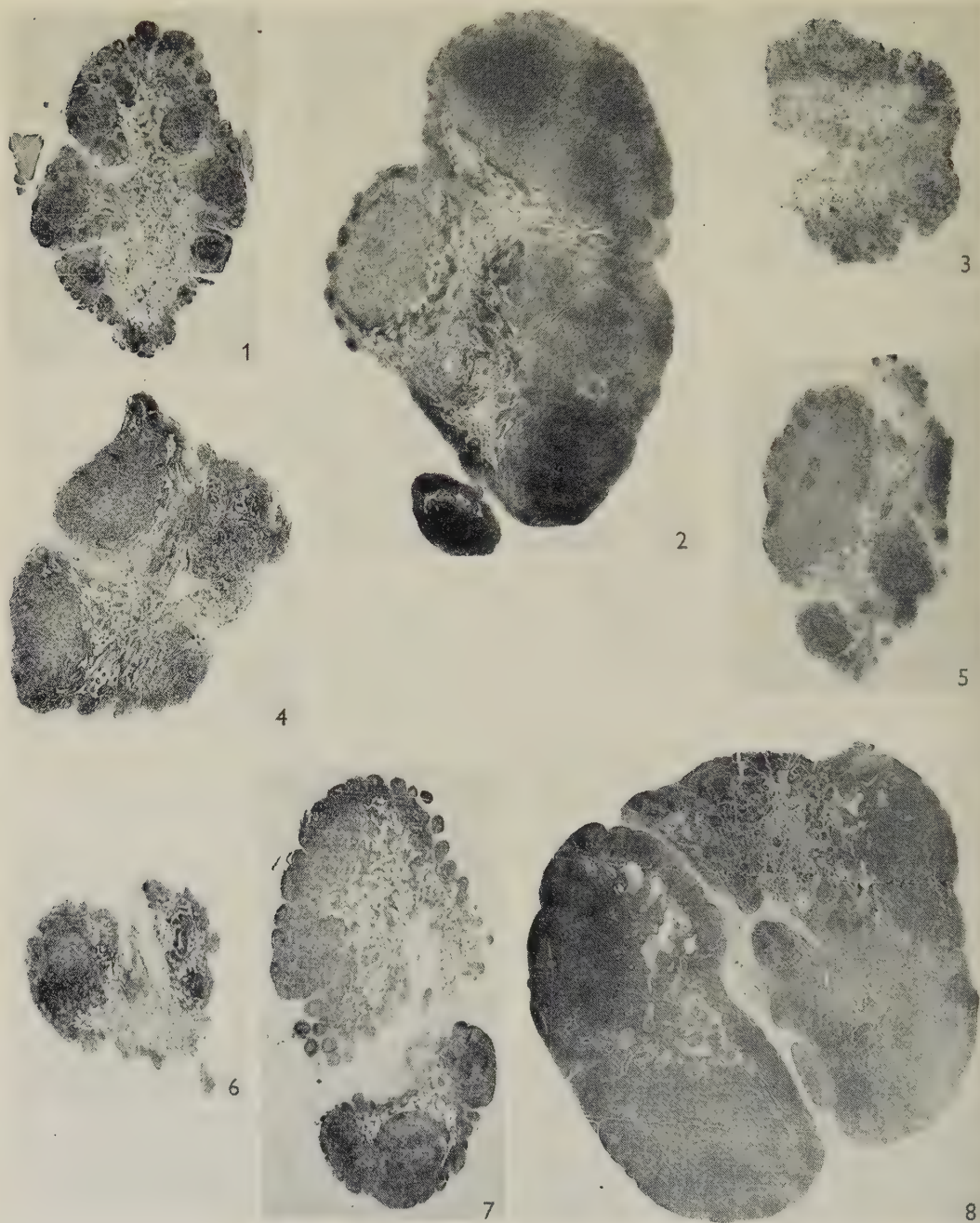
4. The mechanism of action of cortisone in prolonging homograft survival is discussed, and it is tentatively concluded that cortisone is effective principally by reducing the power of the graft to elicit the immune response.

I am grateful to Prof. G. M. Wyburn for criticism of the manuscript, to Mr G. Marshall and Mr A. Campbell for technical assistance, and to the Medical Research Council for their generous gift of cortisone.

REFERENCES

- BILLINGHAM, R. E., BRENT, L. & MEDAWAR, P. B. (1954). Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity. *Proc. roy. Soc. B*, **143**, 58-80.
- BILLINGHAM, R. E., KROHN, P. L. & MEDAWAR, P. B. (1951*a*). Effect of cortisone on survival of skin homografts in rabbits. *Brit. med. J.* **1**, 1157-1164.
- BILLINGHAM, R. E., KROHN, P. L. & MEDAWAR, P. B. (1951*b*). Effect of locally applied cortisone acetate on survival of skin homografts in rabbits. *Brit. med. J.* **2**, 1049-1053.
- BILLINGHAM, R. E. & SPARROW, E. M. (1954). Studies on the nature of immunity to homologous grafted skin, with special reference to the use of pure epidermal grafts. *J. exp. Biol.* **31**, 16-39.
- CAVALLI, M. (1935). Sul comportamento dei linfatici nell' innesto autoplastico della pelle. *Sperimentale*, **89**, 504-508.
- KROHN, P. L. & ZUCKERMAN, A. (1954). The effect of splenectomy on the survival of skin homografts in rabbits and on the response to cortisone. *Brit. J. exp. Path.* **35**, 223-226.
- MEDAWAR, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176-199.
- MITCHISON, N. A. (1954). Passive transfer of transplantation immunity. *Proc. roy. Soc. B*, **142**, 72-87.
- PEARSE, A. G. E. (1953). *Histochemistry*. London: J. and A. Churchill Ltd.





SCOTHORNE—EFFECT OF CORTISONE ON SKIN HOMOGRAFT

(Facing p. 427)

- SCOTHORNE, R. J. & MCGREGOR, I. A. (1953). The vascularization of autografts and homografts of rabbit skin. *J. Anat., Lond.*, **87**, 379-386.
- SCOTHORNE, R. J. & MCGREGOR, I. A. (1955). Cellular changes in lymph nodes and spleen following skin homografting in the rabbit. *J. Anat., Lond.*, **89**, 283-292.
- SCOTHORNE, R. J. & SCOTHORNE, A. W. (1953). Histochemical studies on human skin autografts. *J. Anat., Lond.*, **87**, 22-29.
- SCOTHORNE, R. J. & TOUGH, J. S. (1952). Histochemical studies of human skin autografts and homografts. *Brit. J. plast. Surg.* **5**, 161-170.

EXPLANATION OF PLATE

All figures are of ipsilateral node I, the first regional node draining the operated ear ($\times 7$).

- Fig. 1. Node from unoperated control.
- Fig. 2. Node from R319, 4-day homograft, no cortisone. Note great enlargement of tertiary cortical nodules.
- Fig. 3. R378, 4-day homograft, systemic cortisone, 10 mg. per day. Shows no enlargement of cortex.
- Fig. 4. L371, 4-day homograft, systemic cortisone, 10 mg. per day. Shows slight enlargement of cortex.
- Fig. 5. L358, 6-day homograft, systemic cortisone, 10 mg. per day. Shows slight enlargement of cortex.
- Fig. 6. L346, 16-day homograft, systemic cortisone, 10 mg. per day. The node is markedly atrophic.
- Fig. 7. R407, 7-day homograft, cortisone applied locally to the surface of the graft, 2 mg. every third day. The node is somewhat larger than normal, but its general topography is essentially unchanged.
- Fig. 8. L380, 7-day homograft, cortisone injected subcutaneously between graft and regional node, 2 mg. every third day. The node is greatly enlarged, and resembles nodes from untreated homografted animals.

SOME MORPHOLOGICAL EFFECTS OF LARGE DOSES OF CORTISONE IN THE RABBIT WITH SPECIAL REFERENCE TO THE THYMUS AND APPENDIX

By E. J. FIELD

Anatomy Department, University of Bristol

In 1945 Dougherty & White found that single doses of aqueous adrenal cortical extract or adrenal cortical steroids in oil produced rapid lymphocytic breakdown in mice and rabbits. Adreno-cortical influence over lymphocyte production and destruction is complex, and discrepancies in experimental findings and their interpretation sometimes wide (Yoffey, 1952, p. 172). The present observations are based upon control material collected whilst investigating the effect of cortisone upon the development of rabies in rabbits, and deal mainly with changes in the thymus and the special lymphoid tissue of the appendix and Peyer patches. Reference is made for purposes of comparison to mesenteric and other lymph nodes.

MATERIAL AND METHODS

Young rabbits (1.3–2.0 kg.) have been used. Twenty animals have been treated with cortisone and sixteen used as controls treated with equal volumes of cortisone suspending medium. Five further animals and a like number of controls were in addition vitally stained with trypan blue. Two litters of newborn rabbits (twelve animals in all) have been used. Sex has not affected the results. Cortisone acetate (Merck or Roussel) was given intramuscularly, usually 40 mg./day in two equal doses. Neonatal animals received 10 mg./day. The vitally stained animals were injected with sterile 1% trypan blue solution intravenously on 2 or 3 successive days, the dosage being calculated on the basis of 0.1 g./kg. body weight. Care was taken that experimental and control animals should receive equivalent doses of dye in order that colloidopexic activity in the reticulo-endothelial system might be legitimately compared. All histological material was fixed in Bouin's fluid and in many cases duplicate blocks were fixed by immersion in Zenker's fluid (without formol). For purposes of comparison all photographs shown are of Bouin fixed material.

White blood cell counts were performed before an experiment was begun to rule out the existence of latent infection, and in some of the earlier pairs additional counts were done at intervals after cortisone administration to confirm its efficiency in producing lymphopenia.

RESULTS

Thymus

Changes in the thymus were present *four hours* after a cortisone injection. The nuclei of many cortical thymocytes presented a uniform glassy appearance and stained poorly with haematoxylin. Nuclear chromatin was often margined. Occasional

early ring-like bodies resembling those described in the thymus after irradiation (Schrek, 1948) were found. Changes were strictly limited to the cortex of the thymic lobules. Many mitotic figures were altered, chromosomes being thickened and clumped together resulting in a dense clotted appearance reminiscent of that induced by colchicine (Pl. 1, fig. 1*a-c*), a resemblance remarked upon by Dougherty & White (1945). Often the mitotic process appeared to have been arrested in early telophase (Pl. 1, fig. 1*c*) so that a rounded or oval structure with intensely staining fused chromosome material at either pole was produced. Fragments of nuclear material were often to be seen in the vicinity of disturbed mitoses with which they were apparently associated. Oedema was not a feature of the thymus parenchyma at this or any subsequent stage.

At *seven hours* these changes were more pronounced. Intensely pyknotic cortical thymocytes were found in large numbers, often occurring in islands between which the remaining thymocytes appeared normal. The medulla was unaltered and sharply demarcated from the cortex. Myelocytes were more numerous than in control preparations, especially at the cortico-medullary boundary. 'Pitting' of the cortex, i.e. the production of clear 'punched out' areas, was present and gradually became more marked. By *fourteen hours* changes were widespread in the cortex and contrasted sharply with the normal medulla (Pl. 1, fig. 2). Within the punched-out areas, large pale reticular cells were visible, some containing fragments of nuclear debris (Pl. 1, fig. 3). 'Ringlike' bodies were very numerous and all stages of their transformation into the 'tingible Körper' of Flemming (1885) could readily be followed. The great majority were extracellular at this stage.

By *twenty-four hours* practically no intact cortical thymocytes remained and the boundary between cortex and medulla remained clear. Myelocytes were conspicuous, especially just beneath the cortex. The very few remaining cortical thymocytes resembled those in the medulla and were apparently uninjured. Much cortical debris was still extracellular.

At *thirty-six hours* demarcation between severely damaged cortex and minimally affected medulla was still maintained. Medullary myelocytes continued to be prominent. The whole cortex was reduced to a narrow rim, whilst in the medulla many thymocytes had a more angular form than normal and rather condensed nucleoplasm.

By *forty-eight hours* continuing involution had reduced the thymus to a number of thin strands embedded in loose connective tissue. This tissue was oedematous so that the thymic lobules were more or less widely separated from one another contrasting with the tessellated appearance of a normal gland. The cortex was now made up almost entirely of large pale cells with clear vesicular nuclei. Polymorphs were often found in it and many of the large pale cells showed granules within their cytoplasm. These granules stained mauve with Giemsa, and such cells seemed in process of transformation into myelocytes. Within the medulla there was marked pyknosis of thymocytes and moderate numbers of Flemming bodies had appeared. The changes were qualitatively similar to those seen thirty-six hours earlier in the cortex. The densely staining medulla and very pale thinned cortex gave the thymic lobule an 'inverted' appearance under the low power of the microscope (Pl. 1, fig. 4). Neither at this nor previous stages was anything seen to suggest an active migration of

damaged cells from the cortex into the medulla as described by Dougherty & White (1945). Whilst lymphocytes are, of course, known to possess considerable mobility, it is unlikely that severely damaged cells could migrate in the manner these authors suggest. A vitally stained thymus showed well-laden rounded macrophages in the cortex. These were rounded cells with pale clear rounded nuclei (Pl. 1, fig. 5). Amidst such cells containing large amounts of trypan blue were others apparently similar but having no trace of the dye. A large quantity of nuclear debris still remained uningested by macrophages. No macrophagic activity was visible in the medulla despite the commencing degeneration (Pl. 1, fig. 6). Even at *one hundred and sixty-five hours*, when the animals had been heavily stained with trypan blue, very few cells in the medulla took up vital dye.

The *ninety-six* hour thymus showed large numbers of myelocytes whose granules stained definitely pink. Associated with them was a considerable number of polymorphs with poorly staining granules. It was noteworthy that even when cortisone treatment was pushed up to 180 hr., and the thymus thereby reduced to but a few strands, a certain number of medullary thymocytes remained and appeared normal. The trypan blue stained animals at *one hundred and sixty-five hours* showed very few dye-containing cells in the remaining medulla. The cortex was by then largely cleared of the rounded well-filled macrophages seen earlier. At this stage Hassall's corpuscles appeared enlarged and prominent. A few contained discrete droplets of trypan blue, but the majority stained faintly and diffusely. Many Hassall's corpuscles were crowded with nuclear debris but unstained by vital dye. These late changes in Hassall's corpuscles were especially clear in neonatal animals treated for 12 days with cortisone (Pl. 1, figs. 7, 8; Pl. 2, figs. 9, 10).

Appendix

The structure of the normal rabbit appendix has been described in detail by Entin-knap (1953). He drew attention to the large numbers of bacteria present within the lymphoid tissue of the submucosa, and his description corresponds with that given by Masson & Regaud (1918). Many of the bacteria are engulfed within phagocytes where they undergo lysis. These phagocytes are often grouped at the central pole of the outer of the two follicles usually met with in a transverse section through the appendix. Very clear pictures of extra- and intracellular bacteria can be obtained with Schiff's reagent when cellular nuclei are lightly counterstained with haematoxylin (Pl. 2, fig. 11).

Earliest changes were visible at *four hours* after cortisone injection but were more clear at *seven hours*. Considerable numbers of pyknotic lymphocytes were then found within the cortex of the outer lymphoid follicle, especially in its basal part. Few were present in the innermost part of the lymphoid tissue beneath the mucosa. Many mitotic figures had the clotted appearance referred to above in the description of the thymic changes. Some pyknotic lymphocytes were present in the prominent germinal centres but they were not so numerous as in the cortex of the follicles. Mitotic figures were notably absent throughout this material from the so-called 'germinal centres'.

By *thirteen hours* pyknotic nuclear remains and Flemming 'tingible Körper' had been further increased in number, especially in the cortex of the follicles. As in the

case of the thymus there was a tendency to pitting and the production of small scattered groups of Flemming bodies. But disintegration of lymphocytes was very much less than that seen in the thymus at this stage, the great majority of cells appearing normal. Bacteria-laden phagocytes were prominent and encroached upon the centres of the basal follicles. There was no apparent difference in the proportion of intra- to extracellular organisms.

By *twenty-four hours* the lymphoid tissue had been much reduced in thickness, mainly at the expense of the outer lymphoid follicles which appeared greatly pitted and shrunken, whilst the inner ring of lymphoid tissue was little altered in appearance. By *forty-eight hours* the appendicular wall was clearly thinned to the naked eye, and under the microscope this was seen to be due mainly to the disappearance of lymphocytes from the outermost part of the outer lymphoid follicle, the innermost lymphocytes being normal. Large pale macrophages were prominent in the follicles, especially in their basal parts and 'germinal centres' were enlarged and clear. Bacteria-laden macrophages were numerous and often filled the centres. Under the high power of the microscope the bacteria themselves were seen to be undergoing lysis in normal fashion so that a good deal of intracellular finely granular amorphous material was produced. Trypan blue staining showed the presence of cells loaded with colloid in the subepithelial stroma but none in the basal follicles where lymphocytic disappearance was marked. This absence was strikingly in contrast with the findings in the thymus where trypan stained macrophages were prominent in the cortex of the same animals (though not in the medulla)—see above.

At *ninety-six hours* basal atrophy of lymphoid follicles was more advanced and by *one hundred and sixty-five hours* these parts had practically disappeared. At this stage large lymphocytes appeared with unusual frequency in the subepithelial region.

Peyer patches

No changes were to be seen in this tissue *four hours* after cortisone injection and even at *seven hours* they were minimal, a few mitotic figures presenting a rather 'clotted' appearance. No mitoses were seen in the 'germinal centres' and there was no increase in Flemming bodies. By *fourteen hours* there was an increased number of 'tingible Körper' especially in the basal part of the lymphoid tissue, the lymphocytes immediately beneath the mucous membrane remaining unaltered. Prolongations of lymphocytes from this layer often passed down between the follicles out to the muscular coat. Small, medium and large lymphocytes could readily be made out and mitoses were especially seen amongst the medium-sized cells. By *twenty-four hours* there was only a small increase in the number of Flemming bodies some of which were within the germinal centres. The tempo of change was thus much less than in the appendix. At *forty-eight hours* the lymphoid follicles appeared distinctly smaller, chiefly through basal atrophy. Clear germinal centres were distinct, but there was no striking increase in the number of Flemming bodies. At *seventy-two hours* 'germinal centres' stood out very clearly, and numbers of large lymphocytes with clear vesicular nuclei containing usually two or three 'nucleoli' and possessing very finely granular mauve staining cytoplasm were present (Pl. 2, fig. 12). Such cells occurred also in normal Peyer patches but they appeared more prominent in the stages here described, perhaps as a result of thinning out of small lymphocytes.

There was no evidence of marked lymphocytic destruction and no increase in Flemming bodies.

These large clear cells remained prominent up to the end of observation (*one-hundred and eighty-five hours*). In these later stages the small lymphocytes of the clear germinal centres gradually began to stain more deeply and seemed to return to normal appearance.

Mesenteric, cervical and popliteal lymph nodes

The mesenteric lymph node of the rabbit normally shows a certain degree of oedema of the medulla where numbers of large lymphocytes appear free in the lymph sinuses. Such large cells are sometimes also seen in the subscapular sinus. Considerable, though variable, amounts of pigment giving a faintly positive Schiff reaction and staining green with Giemsa are met with in the reticulum cells of the medulla. Occasional isolated masses of such material may occur in the popliteal lymph node but not in cervical nodes.

Reaction to cortisone though more rapid and marked in the mesenteric than in peripheral nodes, was never of anything like the intensity met with in the thymus. Oedema was not prominent until some *seventy-two hours* of treatment. Large rounded foamy histiocytes described by Dougherty & White (1945, fig. 19) were only occasionally seen in small groups or as isolated cells in mesenteric nodes and not in appreciable numbers in cervical or popliteal nodes. Their presence did not seem to be associated with cortisone treatment. They were, however, akin to the histiocytes found in considerable numbers in the lymphoid follicles of the appendix and Peyer patches, where they were clearly seen to arise from macrophages which had digested bacteria and other cellular debris. Such cells were often finely vacuolated and showed elongated narrow clear channels in their cytoplasm.

'Giant cells' of the type described by Dougherty & White were not seen in the present material.

Spleen

Splenic infiltration with polymorphs and nuclear debris was observed early (*seven-fourteen hours*), and later there was considerable reduction of pulp so that remaining lymphatic nodules stood out clearly and, though small, were remarkably well preserved even after *ninety-six hours* treatment.

DISCUSSION

The nature of the small cells of the thymic cortex is not settled. Whilst the majority of authors accept them as being lymphocytes some maintain strongly that they differ in important respects from true lymphocytes both as regards structure and function (Dustin, 1923). The present results support the view that the small round cells of the thymic cortex are different from lymphocytes found in lymph nodes. It has been known for many years that the thymus is more sensitive to various agencies than are the peripheral lymph nodes (literature reviewed by von Moellendorff, 1940). Dustin (1923) showed very clearly the markedly different response to fasting (now a well-recognized 'stressor') made by the thymus and lymph nodes of the newborn cat. He emphasized the early and punctate reaction in the former as opposed to the sluggish and diffuse changes which occurred much later in the latter. Dougherty

& White (1945) do not emphasize this important difference but find that 'changes in Peyer's patches occurred earlier and were as extensive or more so than the alterations in other lymphoid tissues' (p. 90). On the other hand, Baker, Ingle & Li (1951) found that the thymus was considerably more sensitive to the lytic action of the adrenal steroids than either the lymph nodes or the spleen. They were inclined to associate this greater thymic sensitivity with the facts that cellular proliferation is four to six times more rapid in the thymus than in the spleen and lymph nodes (Andreasen & Christensen, 1949), and that the turnover of desoxyribonucleic acid is four or five times greater. It is now known that cortisone has a catabolic or anti-anabolic effect on protein metabolism (Long, Katzin & Fry, 1940; Hoberman, 1950; Clark, 1950; Silber & Porter, 1953) so that the more pronounced effect of the drug on active sites of lymphocytic production is understandable. Interference with protein turnover diminishes thymocyte and lymphocyte production and also causes morphological degeneration of already formed cells especially in the thymus. Whilst no indisputable morphological differences can be made out in paraffin sections between the small round cells of the thymus and lymphoid tissue elsewhere in the body, the former appear much denser and resist destaining longer than do the latter. This is well seen when a section of thymus together with a juxtathymic lymph node is examined. The pallor of the latter as compared with the deep staining of the thymic cortex is clear, both having been subjected to the same staining procedure.

Careful examination shows that amongst the densely packed small cells of the thymic cortex there are some which can be classed as 'medium' or 'large' and generally these stain more lightly and have a more 'open nucleus'. The small cells appear to be slightly smaller than those of lymphoid tissue elsewhere. Jacobi (1935) has estimated that in the human thymus they measure $4.0-4.5\mu$ as opposed to 4.5μ in lymph nodes. The degeneration which results from cortisone injection affects especially the small thymic cells. This accords with Dustin's (1923) well-reasoned view that the smallest thymocytes—those which have arrived at the 'stade terminale d'élassosis'—are most sensitive, whilst the larger ones tend to escape. The medullary cells being less mature are less affected. Degeneration 'en foyers' is well marked and Dustin's explanation that the destruction affects groups of cells which are of the same age—'groupements isogéniques'—seems acceptable.

Within the appendix, too, not all lymphocytes of the submucous follicles are equally sensitive to cortisone, even though they are morphologically uniform. There would appear to be fine biochemical differences which common histological methods do not bring out.

Ringerz, Fagraeus & Befglund (1952) reported on the effects of a single dose (1 mg. intra-muscularly) of cortisone on the mouse (15 g.). They found early and severe changes in the cortex of the thymus and relatively slight changes in the medulla. They do not regard the cortical thymic cells as more sensitive to cortisone than those of the medulla or lymph nodes, but believe that the cortex of the thymus is mainly a receptacle for lymphocytes and lacks the capacity of continually compensating through regeneration. It may be noted, however, that mitotic figures are numerous in cortical thymocytes.

Whilst Ringerz *et al.* limited their investigation in the mouse to single doses of cortisone, Weir & Heinle (1950) gave daily subcutaneous doses of 1 mg. to mice for

up to thirty days. Whilst they found that the spleens of such animals were reduced to about half their normal size, the lymph nodes and thymus glands were normal both grossly and histologically. Yoffey & Baxter (1946) noted slight but definite hyperplastic changes in lymphoid tissue when adult rats were injected daily with aqueous adrenal cortical extract over 4 weeks. Such results emphasize once again the importance of species differences and design of experiment in assessing the effect of cortisone and adrenal extracts upon lymphoid tissue.

Granular eosinophile cells have long been known to occur in the thymus of a variety of animals including the rabbit (Maximow, 1909). There has been some confusion in the past between such cells and mast cells which are, however, almost entirely limited to the so-called 'capsule' of the gland and its interlobular septal prolongations. They appear increased in number in the interlobular septa of a degenerated thymus owing to general shrinkage of the organ. No alteration in their character has been observed. True eosinophile cells, on the other hand, occur within the parenchyma of the thymus. Their origin has been claimed to be from the blood or locally from a small cell of the thymic cortex. An extensive literature relating to this question is reviewed in Hammar's monograph (1936) and by Bargmann (1943). So far as the present material is concerned there seems no doubt that some of the small and large thymic cells can acquire eosinophilic granules in gradually increasing numbers, meanwhile retaining their lymphocytic type of nucleus (Pl. 2, fig. 13) until a 'myelocyte' not much larger than a lymphocyte, is produced. Some nuclei then develop an indentation. The sequence makes it reasonably sure that the origin of the eosinophilic myelocytes is local within the thymus parenchyma. There is, moreover, no evidence that eosinophilic granules are really fragments of degenerated red cells as has been maintained by some authors (Barbano, 1912). Amongst more recent workers Jordan & Looper (1928) found clear evidence in the box-turtle of transformation of small thymic cells into granulocytes. They found the earliest granules to be small, spherical and basophilic later becoming larger and eosinophilic. Similarly, Bloom (1937) who observed the transformation of rabbit thoracic duct lymphocytes into granulocytes in tissue culture, found the earliest granules to stain purple later becoming eosinophilic. This same sequence was seen in the present material in specimens where their increased granulocyte production was associated with severe retrogressive changes in the thymus gland. It is possible that such changes are themselves a chemical stimulus to leucocyte accumulation and that local sources respond actively.

Appendix

Macrophages. Animals treated with cortisone showed increased numbers of bacteria-laden macrophages within the lymph follicles. This increase might be due to (a) a failure of the laden cells to migrate in the normal way out of the follicles, (b) chemotaxis by lymphocytic degeneration products or by increased numbers of bacteria within the follicles, (c) diminished phagocytic capacity of individual macrophages so that more cells were required to deal with the organisms. It might also be to some extent apparent rather than real because of general shrinkage of the lymphoid follicles. This cannot be the sole cause since there is also a different distribution of the phagocytes in treated animals (see above). Many lymphocytic particles

remain extracellular both in the appendix and in the thymus for long periods, gradually disappearing by lysis without intracellular digestion. This is clearly seen in vitally stained animals. In fact, the number of phagocytes in the thymus seems inadequate to clear the great destruction which occurs and much seems to be accomplished extracellularly. It is known that cortisone depresses the mobility of phagocytic cells (Paff & Stewart, 1953) so that accumulation to the appendicular follicles might in part be due to inability of laden cells to emigrate. The individual cells are well filled with bacteria and no significant difference in the degree of intracellular digestion could be made out.

Whilst no systematic attempt was made to study the regeneration process in lymphoid tissue following injection of cortisone, in two rabbits portions of the appendix were removed at the end of cortisone injection and again after intervals of some weeks had elapsed to gain some idea of the restorative capacity of the local lymphoid tissue. Six weeks after cessation of treatment the much thinned appendicular wall had reverted to its normal thickness. Histologically the lymphocytes were a little sparse in the follicles which were, however, otherwise normal.

'Tingible Körper' (Flemming bodies)

These rounded deeply staining and often vesicular bodies were described by Flemming (1885) as occurring chiefly in the germinal centres of lymph follicles, and for the most part within the cytoplasm of phagocytes. They are generally recognized to be especially numerous in the vicinity of large numbers of mitotic figures (Bloom, 1938; de Bruyn, 1948; Yoffey, 1950). The association between 'tingible Körper' and mitotic activity was clearly stated by Jolly (1923). He pointed out that 'à côté des figures de division cellulaires, les centres germinatives montrent de nombreuses figures de destruction nucléaire' in the form of Flemming bodies; and again 'ces phénomènes de dégénérescence nucléaire, parfois très marqués, peuvent se voir dans le même centre, à côté de nombreuses mitoses'. In the present material considerable numbers of Flemming bodies were found in association with lymphocytic mitosis. It seemed in many cases that Flemming bodies had been formed during the process of mitosis, either by ejection of nuclear masses or by condensation and/or fragmentation in metaphase. The ratio between Flemming bodies and mitotic figures was determined in normal and in cortisone-treated animals for the appendix, Peyer patch and cervical lymph node. These data have been analysed statistically by Dr G. Herdan in an appendix to this paper, where it is shown that there is a significant increase in the ratio of Flemming bodies to mitotic figures after injection of cortisone.

Dissolution of lymphocytic cytoplasm

Dougherty & White (1945) studied the process of lymphocytic dissolution following cortisone administration by the air-dried imprint method. They found two types of degeneration: (a) denuded lymphocyte nuclei, (b) budding of cytoplasm. They believed that there was a true accentuation of the budding process in experimental lymph nodes since the number of cells with buds was much greater than normal. Yoffey (1950) has criticized this morphological evidence, and remarks that Downey & Weidenreich's (1912) observations upon the budding process as seen in sections

requires confirmation. The present sectioned material was therefore examined carefully for evidence of lymphocytic budding, both in normal and experimental animals. Very rare examples of clearly outlined buds were found in one or two treated glands, especially in the medulla or subscapular sinus of mesenteric nodes (Pl. 2, fig. 14), but nothing convincing in normal or control glands. On the other hand, lymphocytes both small and large with ragged cytoplasm were more common in the mesenteric nodes of treated animals. Since lymphocytes of the medulla may be formed from reticular cells (Downey & Weidenreich, 1912; Sundberg & Downey, 1942) by a process of detachment from the syncytium and rounding up, it is possible that mechanical forces resulting from oedema or later shrinkage of parenchyma may produce unusually large numbers of ragged lymphocytes in experimental glands.

Altogether, no convincing evidence for a marked increase in true cytoplasmic budding from degenerating lymphocytes was found. Undoubtedly the most marked feature of lymphocytic dissolution is a nuclear change.

STATISTICAL ANALYSIS AND DISCUSSION

The results of the experiment have been summarized in the following table of averages:

	Control				Experiment				<i>t</i>	<i>P</i>
	Exp.	Mitosis	Flemming bodies	Flemming bodies/mitosis	Exp.	Mitosis	Flemming bodies	Flemming bodies/mitosis		
Appendix	520	15.35	30.35	2.12	521	11.6	101.05	9.65	6.2	≤0.01
	526	8.6	7.9	0.91	527	3.4	18.3	5.51	8.5	≤0.01
	525	10.6	7.8	0.75	523	7.72	43.8	7.30	5.14	≤0.01
Lymph nodes	520	9.33	25.8	3.01	521	4.33	38.3	10.29	2.68	<0.05
	525	3.25	7.13	2.34	523	2.5	14.74	6.73	4.34	≤0.05
Peyer patch	520	13.7	24.5	1.79	521	4.6	30.7	6.97	9.04	≤0.01
	525	11.7	12.1	1.09	523	8.4	15.3	1.97	3.22	≤0.05

Inspection of the above table shows that there is an effect of treatment on both mitosis and Flemming bodies. In every site which was investigated it depresses the mitosis and raises the number of Flemming bodies. It follows that the increase in the ratio Flemming bodies/mitosis produced by the treatment is due to both the increase in the numerator and the decrease in the denominator. The statistical significance test applied to the average ratios leaves little doubt that the increase of that quantity is real and due to the treatment.

SUMMARY

1. Injection of cortisone acetate suspensions into young adult rabbits produced degenerative changes in the lymphoid tissues, commencing about 4 hr. after injection.

2. These changes were very much earlier and more severe in the thymus than in any other tissue. Within the thymus there was in the early stages a punctate degeneration of cortical thymocytes. The significance of this is discussed with special reference to the nature of thymocytes. Hypertrophy of Hassall's corpuscles occurred after more prolonged administration of cortisone.

3. Changes in the appendix were more severe than those in Peyer's patches and these latter more marked than in mesenteric, cervical or popliteal lymph nodes.

4. Lymphocytic degeneration was due partly to interference with mitosis producing a 'clotting' and also of direct action causing disintegration of the nucleus. There was considerable variation in the susceptibility to cortisone of morphologically identical 'lymphocytes'.

5. No convincing evidence of cytoplasmic budding as a stage in lymphocytic degeneration was found.

6. No evidence of altered colloidopexic activity of the reticulo-endothelial system *vis-à-vis* trypan blue was observed under the influence of cortisone; particles of disintegrated thymocytes may disappear without intracellular digestion; cortisone does not interfere with normal phagocytosis of bacteria within appendicular lymph follicles, but may hinder migration of phagocytes.

The author is grateful to Prof. J. M. Yoffey for helpful suggestions both as regards material and literature. He would also like to thank Dr G. Herdan for the statistical analysis of results.

He is indebted to Mrs C. M. Bruce for the preparation of histological sections and to Drs C. H. G. Price and G. M. Jeffree for blood and marrow examinations carried out in the earlier part of the investigation. The author is also grateful to the Medical Research Council for the provision of cortisone and for defraying the cost of the work.

REFERENCES

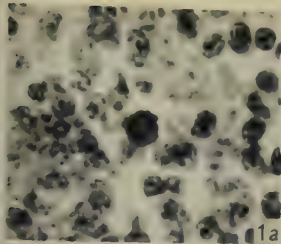
- ANDREASEN, F. & CHRISTENSEN, S. (1949). The rate of mitotic activity in the lymphoid organs of the rat. *Anat. Rec.* **103**, 401-412.
- BAKER, B. L., INGLE, D. J. & LI, C. H. (1951). The histology of the lymphoid organs of rats treated with adrenocorticotropin. *Amer. J. Anat.* **88**, 313-349.
- BARBANO (1912). Die normale Involution der Thymus. *Virchows Arch.* **207**, 1-27.
- BARGMANN, W. (1943). *Der Thymus*, in von Möllendorff's *Handbuch der mikroskopischen Anatomie des Menschen*, vi, 4. Springer. Berlin.
- BLOOM, W. (1937). Transformation of lymphocytes into granulocytes in vitro. *Anat. Rec.* **69**, 99-121.
- CLARK, I. (1950). Effect of cortisone on protein metabolism in rats studied with isotopic glycine. *Fed. Proc.* **9**, 161.
- DE BRUYN, P. P. H. (1948). The effect of X-rays on the lymphatic nodule, with reference to the dose and relative sensitivities of different species. *Anat. Rec.* **101**, 373-405.
- DOUGHERTY, T. F. & WHITE, A. (1945). Functional alterations in lymphoid tissue induced by adrenal cortical secretion. *Amer. J. Anat.* **77**, 81-116.
- DOWNEY, H. & WEIDENREICH, F. (1912). Ueber die Bildung der Lymphocyten in Lymphdrüsen und Milz. *Arch. mikr. Anat.* **80** (Abt. 1), 306-394.
- DUSTIN, A. P. (1923). Thymocytes et lymphocytes. Demonstration experimentale de leurs différences de potentialité. *Ref. franc. Endocr.* **1**, 332-345.
- ENTIKNAP, J. B. (1935). Phagocytosis of intestinal bacteria in the appendix of normal rabbits. *J. comp. Path.* **63**, 7-14.
- FLEMMING, W. (1885). Studien über Regeneration der Gewebe. *Arch. mikr. Anat.* **24**, 50-91.
- HAMMAR, J. A. (1936). *Die Normal-Morphologische Thymusforschung*. Barth: Leipzig.
- HOBERMAN, H. D. (1950). Endocrine regulation of amino acid and protein metabolism during fasting. *Yale J. Biol. Med.* **22**, 341-367.
- JACOBI, W. (1935). Die Zellkerngrösse beim Menschen. *Z. mikr.-anat. Forsch.* **38**, 161-240.
- JORDAN, H. E. & LOOPER, J. B. (1928). The histology of the thymus gland of the Box-Turtle, *Terrapene Carolina*, with special reference to the concentric corpuscles of Hassall and the eosinophilic granulocytes. *Anat. Rec.* **40**, 309-337.

- LONG, C. N. H., KATZIN, B. & FRY, E. G. (1940). The adrenal cortex and carbohydrate metabolism. *Endocrinology*, **26**, 309-344.
- MASSON, P. & REGAUD, CH. (1918). Sur l'existence de nombreux microbes vivant a l'état normal dans le tissu des follicules lymphoïdes de l'intestin chez le lapin. *C.R. Soc. Biol., Paris*, **81**, 1256-1270.
- MAXIMOW, A. (1909). Untersuchungen über Blut und Bindegewebe. II. Über die Histogenese der Thymus bei Säugetieren. *Arch. mikr. Anat.* **74**, 525-621.
- PAFF, G. H. & STEWART, R. (1953). Free wandering cells and cortisone. *Proc. Soc. exp. Biol., N.Y.*, **83**, 591-592.
- RINGERZ, N., FAGRAEUS, A. & BERGLUND, R. (1952). On the action of cortisone on the thymus and lymph nodes in mice. *Act. path. microbiol. Scand.* **30** (Suppl. 93), 44-51.
- SCHREK, R. (1948). Cytologic changes in thymic glands exposed in vivo to X-ray. *Amer. J. Path.* **24**, 1055-1063.
- SILBER, R. H. & PORTER, C. C. (1953). Nitrogen balance, liver protein repletion and body composition of cortisone treated rats. *Endocrinology*, **52**, 518-525.
- SUNDBERG, R. D. & DOWNEY, H. (1942). Comparison of lymphoid cells of bone marrow and lymph nodes of rabbits and guinea pigs. *Amer. J. Anat.* **60**, 455-597.
- WEIR, D. R. & HEINLE, R. W. (1950). Similarity of hematologic effect of pyridoxine deficiency cortisone and myeloid metaplasia factor of human urine. *Proc. Soc. exp. Biol., N.Y.*, **75**, 655-658.
- YOFFEY, J. M. (1950). The mammalian lymphocyte. *Biol. Rev.* **25**, 314-343.
- YOFFEY, J. M. (1952). In *The Suprarenal Cortex*; proceedings of the Fifth Symposium of the Colston Research Society held in the University of Bristol. Butterworth.
- YOFFEY, J. M. & BAXTER, J. S. (1946). Some effects of pituitary adrenotropic hormone (PATH) extract of suprarenal cortex and colchicine on the haemopoietic system. *J. Anat., Lond.*, **80**, 132-138.

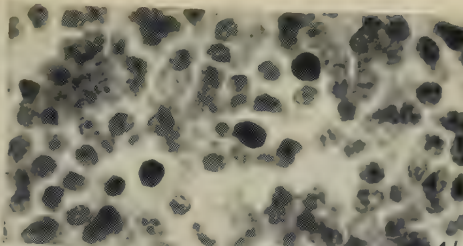
EXPLANATION OF PLATES

PLATE I

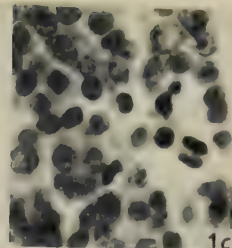
- Fig. 1. (a) Exp. 545. Normal control. Note normal appearance of mitosis. The mitotic figures are larger and individual chromosomes can often be made out. Wohlbach-Giemsa. $\times 850$. (b) Exp. 522. 4 hr. Cortex of thymic lobule. Note several 'clotted' mitoses in thymocytes. The individual chromosomes are not visible and the retracted mass of nuclear material stains very deeply. Wohlbach-Giemsa. $\times 850$. (c) Exp. 522. 4 hr. Note group of 'tingible Körper'. Below and to the left is a Flemming body apparently produced by condensation of nuclear material in early telophase. A smaller similar structure is seen above and to the right. Wohlbach-Giemsa. $\times 850$.
- Fig. 2. Exp. 523. 14 hr. Thymus lobule. Note clear demarcation of cortex from medulla and the 'pitted' appearance of the cortex. $\times 94$.
- Fig. 3. Exp. 529. 14 hr. Thymus lobule cortex. The clear 'pits' in the cortex are occupied by large phagocytic cells. Note the clear vesicular nucleus. The Flemming bodies are mainly extracellular. Many thymocytes are normal. $\times 825$.
- Fig. 4. Exp. 536. 48 hr. Thymus lobule. Note 'inverted' appearance - the medulla appearing relatively more dense than the cortex. $\times 85$.
- Fig. 5. Exp. 563. 48 hr. Thymus cortex. Vitally stained animal. Note large rounded macrophages containing many globules of trypan blue. Groups of Flemming bodies are present and are for the most part extracellular. A number of phagocytes have not taken up the vital dye. $\times 900$.
- Fig. 6. Exp. 563. Thymus medulla of same specimen as fig. 9. Despite numerous Flemming bodies there are no trypan blue stained macrophages to be seen. $\times 990$.
- Fig. 7. Exp. 559D. Hassall's corpuscles in 10-day-old control rabbit. This is an unusually large corpuscle. A small amount of nuclear debris is commonly present in the corpuscles. On the right is an early corpuscle. $\times 370$.
- Fig. 8. Exp. 559C. Hassall's corpuscles from treated animal (see legend above). Note large size of the corpuscle and numerous nuclear fragments within it. Despite the extensive changes many thymocytes appear normal. $\times 370$.



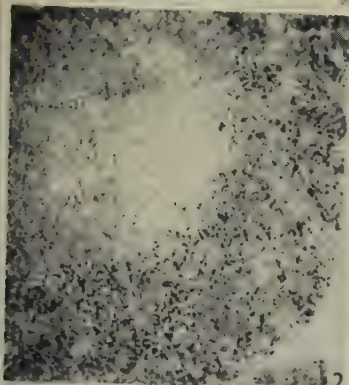
1a



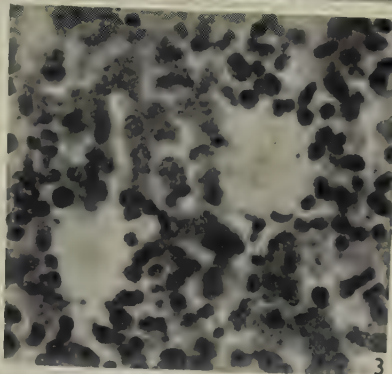
1b



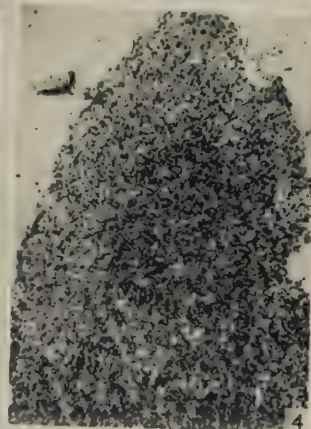
1c



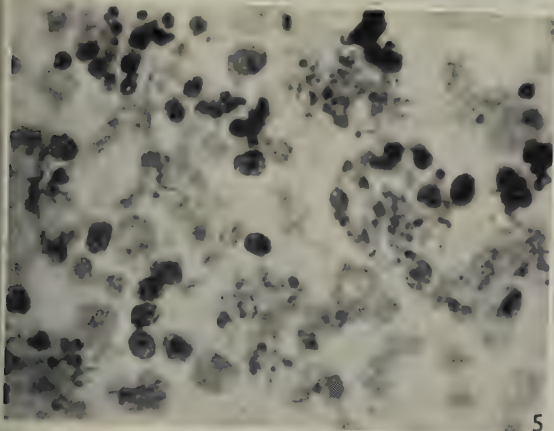
2



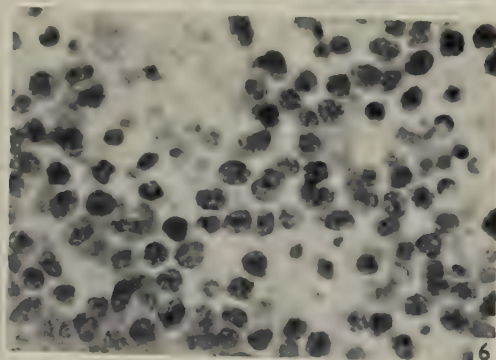
3



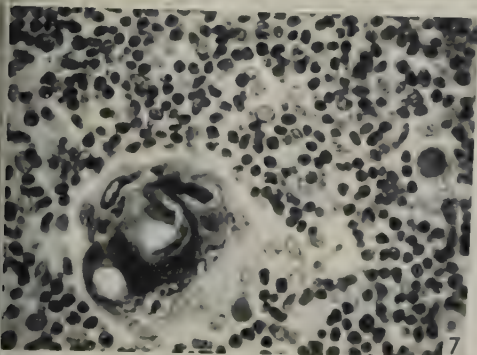
4



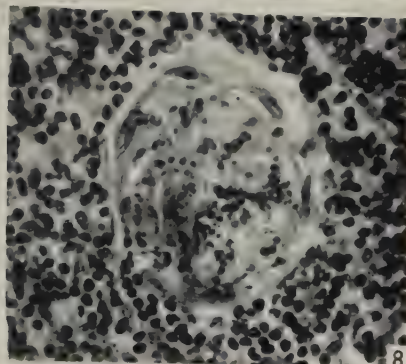
5



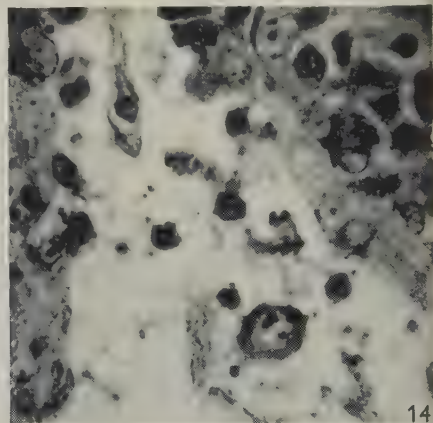
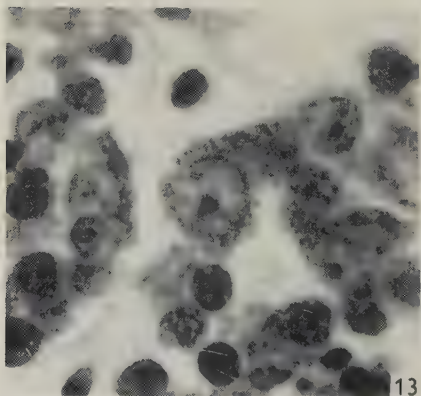
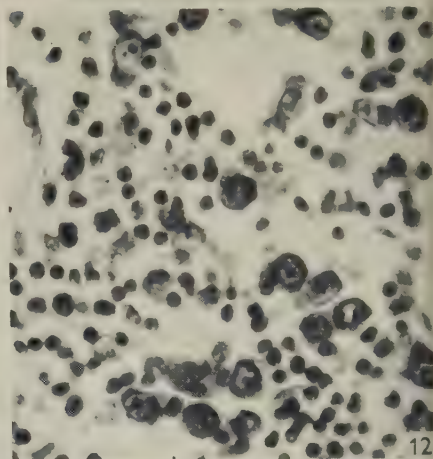
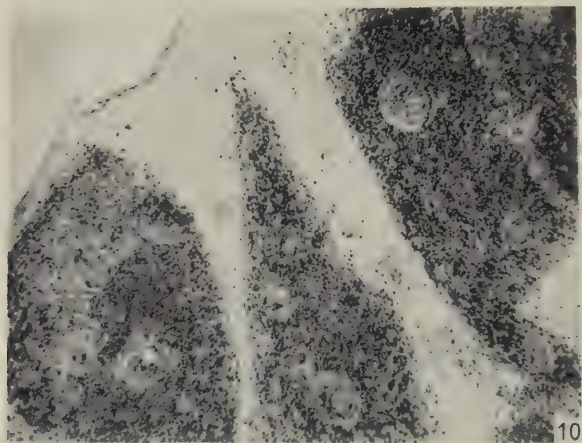
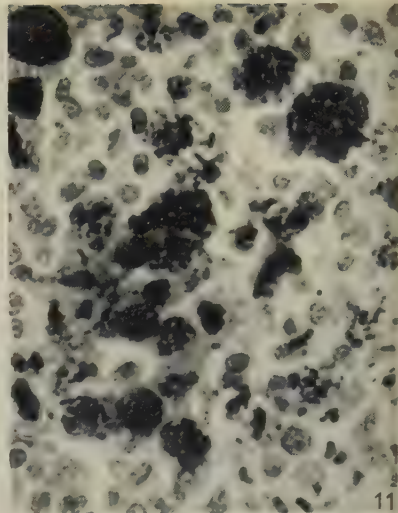
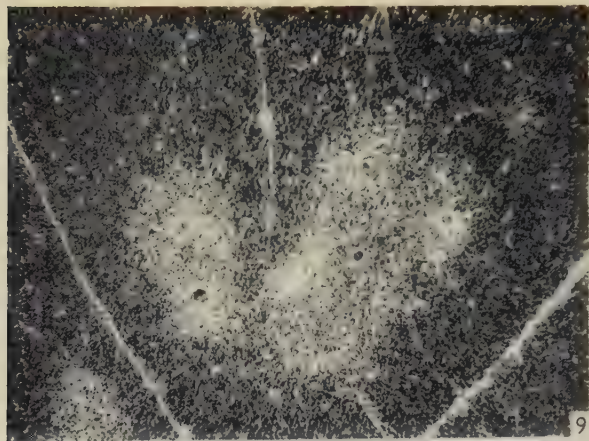
6



7



8



FIELD —MORPHOLOGICAL EFFECTS OF CORTISONE IN THE RABBIT

PLATE 2

- Fig. 9. Exp. 559D. Thymus of 10-day-old control rabbit. Note clearly differentiated cortex and medulla within each lobule and the relatively small Hassall's corpuscles. $\times 73$.
- Fig. 10. Exp. 599C. Thymus of 10-day-old litter mate of Exp. 559D which has received 2.0 ml. (50 mg.) cortisone in the first 8 days of life. Note high degree of involution of the thymus with obliteration of the cortico-medullary boundary and large Hassall's corpuscles. $\times 73$.
- Fig. 11. Exp. 539. Normal appendix. Note macrophages containing large numbers of bacteria and also considerable numbers of extracellular bacteria. Schiff stain counterstained with haematoxylin. $\times 610$.
- Fig. 12. Exp. 546. 72 hr. Peyer patch. Considerable numbers of large lymphocytes are present. They possess one or two 'nucleoli' in a pale vesicular nucleus and have distinct deeply staining cytoplasm. Wohlbach-Giemsa. $\times 550$.
- Fig. 13. Exp. 537. 7 hr. Thymus cortex. Large lymphocyte developing mauve staining granules in its cytoplasm. Wohlbach-Giemsa. $\times 1575$.
- Fig. 14. 72 hr. Peripheral sinus of mesenteric lymph node. Note large and several small lymphocytes showing cytoplasmic 'buds'. Wohlbach-Giemsa. $\times 820$.

THE HISTOCHEMICAL DISTRIBUTION OF 'LIPASE' IN THE ALIMENTARY TRACT AND ASSOCIATED GLANDS OF LABORATORY ANIMALS

BY B. F. MARTIN

Department of Anatomy, The University, Sheffield

INTRODUCTION

Several methods now exist for the histochemical demonstration of esterolytic enzymes. The first method, known as the 'Tween' technique, was introduced by Gomori (1945). Subsequently, an 'azo dye' method was evolved by Nachlas & Seligman (1949*a*), patterned on that originally introduced for alkaline phosphatase by Menten, Junge & Green (1944). A third technique has been described independently by Barnett & Seligman (1951) and by Holt (1952). This depends on the hydrolysis of indoxyl derivatives to indigo at the sites of enzyme activity. Although the early results were rather crude, this technique has recently been brought to one of considerable refinement by Holt (1954), whose illustrations show that a very discrete localization of enzyme activity is obtained. It seems likely that this technique will prove superior to the other two.

Subsequent to the introduction of the 'Tween' technique, reports of large-scale studies employing this technique are few; in fact, the only really major study is that of Gomori himself (1946), whose report was rather exhaustive, and the paucity of later work can probably be attributed to the difficulty encountered in obtaining consistent results. The present work comprises a comparative study using a modification of the 'Tween' technique which gave more consistent and precise results (Martin, 1953*a*).

In the first place, it is necessary to consider what esterolytic enzyme or group of enzymes are histochemically demonstrable with this and the allied techniques. At the present time, enzymes operative upon esters of the carboxylic acids are divided into three groups, viz. true lipase, non-specific esterase and cholinesterase. Cholinesterase stands in a special class from the other two, in that it hydrolyses choline esters at a rapid rate, and is inhibited by minute amounts of eserine. Even this group is divisible into true and non-specific cholinesterase; the former has a limited distribution, chiefly in nerve cells and plexuses, motor end-plates and mammalian red blood corpuscles, whilst the latter is more widespread and attacks simple aliphatic as well as choline esters, and may occur in association with non-specific esterase.

From the time the term lipase was first used by Hanriot (1896) to designate an enzyme which he found in blood serum and in a number of tissues, there has been indiscriminate use of the terms lipase and esterase. In recent years, emphasis has been laid on the need for more accurate use of terminology. It is now understood that distinction between lipase and non-specific esterase may be made in two ways,

namely, according to the chain length of the fatty acid esters which are the more selectively attacked, and secondly by the influence of activators and inhibitors upon the enzyme activity. Although there is some degree of overlap in their activity, especially towards esters of intermediate chain length, lipase attacks predominantly long chain esters whilst non-specific esterase reacts with short chain esters. Many substances are known to influence the activity of these enzymes; for example, lipase is activated by bile salts and calcium oleate, but is inhibited by quinine, whilst non-specific esterase is inhibited by bile salts, fluoride and atoxyl.

Results with the 'Tween' technique were originally classified as due to lipase activity, but although the 'Tweens' are long-chain saturated fatty acid esters (of polymannitols and polyglycols), the histochemical results show that they are attacked by non-specific esterase as well as lipase, and this is probably due to their special constitution which makes them water soluble (Nachlas & Seligman, 1949*c*). True lipase has a very limited distribution, i.e. in the pancreatic acini and, in certain species, in the gastric glands.

The 'azo dye' and 'Indigo' techniques differ from the 'Tween' technique in that they demonstrate sites of cholinesterase activity as well as those of lipase and non-specific esterase. In the present work, the term 'lipase' has been adopted to indicate the enzyme activity demonstrated by the 'Tween' technique, but it is used in a generic sense to embrace the lipase/non-specific esterase group of enzymes.

MATERIALS AND METHODS

A comparative histochemical study was made of the salivary glands, oesophagus, stomach, pyloro-duodenal junction, small intestine, large intestine, pancreas, liver and gall bladder, and the species studied were dogs (8), cats (8) guinea-pigs (6), rabbits (6), rats (5) and mice (5).

Thin pieces of the tissues were fixed in absolute acetone in the refrigerator overnight, dehydrated through two changes of absolute acetone, cleared in two changes of benzene (about half an hour each) and impregnated with paraffin for 2 hr. at 56° C. Serial sections were cut at a thickness of 5 μ and flattened on water which must be lukewarm (35° C) because of their tendency to fragment at higher temperatures.

The histochemical technique employed was essentially Gomori's (1951) modification of his original (1945) technique. Briefly, the procedure is as follows. After the sections have been dewaxed in xylol and taken through descending grades of alcohol to water, they are incubated at 37° C. for 6-24 hr. or even longer (depending upon the activity of the tissue) in a substrate bath containing the 'Tween' compound, together with calcium chloride and glycerol (the latter being a slight enzyme activator), made up in a barbital buffer at approximately pH 7.4. 'Tween' 40 (a palmitic ester) was used for the present investigation. At the sites of enzyme activity, calcium ions combine with the fatty acid liberated from the 'Tween' to form an insoluble colourless salt: this is converted to the lead salt by immersion of the slides in lead nitrate after washing them in distilled water. After further washing, immersion of the slides in ammonium sulphide converts the lead salt into a golden brown precipitate of lead sulphide. Control sections are treated for one minute with Lugol's iodine before incubation, which effectively destroys the enzyme

activity. The reaction is sharply confined to the cytoplasm of cells and is never seen in nuclei, either with this or the allied techniques. Nuclei can therefore be counterstained with haematoxylin (e.g. Harris's) before the slides are dehydrated, cleared and mounted. Unfortunately the precipitate is difficult to preserve. As Gomori has pointed out, xylol must be avoided as the clearing agent or solvent for the mounting medium: he advised clearing in tetrachloroethylene and mounting in clarite dissolved in the same solvent.

In the present study, some sections of each series were cleared and mounted in this way, though only clarite substitute was available, and others were mounted in glycerine jelly direct from water. Although both methods prevent early fading, most sections do fade in time with the formation of crystals scattered irregularly over the section. It is advisable therefore to interpret sections soon after their preparation, and to take representative photomicrographs.

In the early stages of this investigation, the results were somewhat disappointing. Sections from the same tissue block frequently gave different intensities of reaction, and where the reaction was a weak one, some sections might give none at all.

A procedure was devised which gave more consistent and precise results (Martin, 1953*a*); a similar procedure was independently described by Richterich (1952*a*). The sections are introduced directly into the substrate bath without dewaxing (or any other prior treatment), and after incubation they are passed through the usual steps; even counterstaining of the nuclei can be effected satisfactorily in spite of the paraffin coat. After dehydration, the paraffin is removed from the sections with tetrachloroethylene, and they are either mounted in clarite substitute or taken back to water and mounted in glycerine jelly. The difference in degree of intensity of the reaction in waxed compared with dewaxed sections is usually quite marked (compare fig. 7 with fig. 8, Pl. 1). Furthermore, there is consistency in the results given by the sections from any one tissue block, and localization is sharp, i.e., there is no scattering of the precipitate outside cell boundaries. This procedure of incubating sections in their paraffin coat was suggested by the work of Ruyter & Neumann (1949), who employed it for another purpose, namely, in an attempt to prevent diffusion artifacts in the histochemical technique for alkaline phosphatase.

RESULTS

Although the distribution of 'lipase' was found histochemically to be constant in any one species, individual variation in the intensity of the reaction was a noteworthy feature. Authors of histochemical studies do not seem to have drawn attention to this point, yet in the past several biochemists have commented on the variable esterolytic activity of organs they studied (e.g. Loevenhart, 1902; Virtanen & Suomalainen, 1933). It has also been observed that the serum esterase (of man) varies considerably in different healthy persons, and from time to time in the same subject, without any obvious external cause. It is not apparently influenced by such factors as food ingestion or exercise, and is not related to sex, age or weight (Lagerlöf, 1945).

Although in the present work no special study was undertaken to determine what factors influence the activity of the enzyme, there was no obvious relationship between the age, sex, or digestive state of the animal and the histochemical reaction

in the tissues. It must be emphasized, however, that in view of this variation in intensity of the reaction, organs from a number of animals of each species must be studied, otherwise some sites of activity may not be brought to light.

The results of this comparative study are presented organ by organ and are equated with Gomori's (1946) account, which included observations on the dog, rabbit, guinea-pig and rat, and is the major existing report on results obtained with the 'Tween' technique. Comparison is also made with the 'azo dye' results of Nachlas & Seligman (1949*b*) for the dog, rabbit, guinea-pig and rat, and with those of Chessick (1953) for the cat, rabbit, rat and mouse. Large scale comparative studies with the improved 'Indigo' method of Holt (1954) are as yet lacking, but with the older method the results are similar to those with the azo dye (see Barnett, 1952).

The pancreas

In all species studied, a strong reaction was given by the acinar cells, principally in their apical parts. A patchy reaction was commonly obtained in the lining epithelium of the larger ducts and the secretion within their lumen was strongly positive, which is in keeping with the fact that the pancreatic juice contains true lipase. Pl. 1, fig. 1, illustrates the typical picture, as seen in cat pancreas.

The islets (of Langerhans) were negative in the cat, dog and rat, but in the rabbit, guinea-pig and mouse, a reaction in some of the islet cells was seen, though not in all specimens. A common feature of the pancreas was that the walls of arteries were often positive in the rabbit, guinea-pig and rat, the reaction being present in the muscle coat and, in the rat, sometimes in the internal elastic lamina also. Positive connective tissue strands around vessels and ducts were seen in rabbit, guinea-pig, rat and mouse.

Gomori (1946) found an intense reaction in the pancreatic acini and in the secretion within the ducts, but he did not report reactions in duct epithelium, islets and connective tissue. However, these sites have been noted to be positive with the 'azo dye' technique (Nachlas & Seligman, 1949*b*; Chessick, 1953), though Chessick regards them as an artifact.

The liver

In all species examined, the cells of the liver lobules gave a very strong reaction, but as with other tissues, there was individual variation in the degree of intensity. Commonly the reaction was evenly distributed throughout the hepatic lobules, but in some cases the reaction was stronger in their central parts. The lining epithelium of the intrahepatic bile ducts was positive in some animals of every species studied, but it was always weak and patchy. The duct contents were invariably negative.

Gomori's (1946) findings were similar, except that he found a distinct reaction in the intrahepatic bile ducts of the rat only. The 'azo dye' results of Nachlas & Seligman (1949*b*) and Chessick (1953) were also similar to the above, but in addition to an intense reaction in the hepatic cells and a weak reaction in the bile ducts, there was a weak reaction in the Kupffer cells in most cases.

The gall bladder

No reports are available for the distribution of 'lipase' in this organ as revealed by the 'Tween' technique. In the present investigation, all the species studied (except the rat, which has no gall bladder but has 'lipase'-positive bile ducts) gave a reaction in the lining epithelium of the gall bladder, but it was always weak and patchy, and the contained bile was invariably negative (Pl. 1, fig. 2). This accords with the picture in the intrahepatic bile ducts. The fact that bile was histochemically negative fits in with Loevenhart's (1902) biochemical observation that bile shows no esterolytic activity.

The 'azo dye' technique also reveals a reaction in the lining epithelium of the gall bladder of some species (rabbit, guinea-pig and dog), and some species also show a reaction in the muscle bundles and in the tunica propria (Nachlas & Seligman, 1949*b*).

The oesophagus

All specimens of this organ were removed from the lower part, close to the stomach. In every species studied the lining epithelium gave a reaction, and this was the only site amongst all the organs examined in which there was a different localization of the precipitate in sections which had been dewaxed as compared with those which were not dewaxed prior to incubation. In the latter sections the reaction was usually decidedly patchy and the reacting groups of cells were located in either the superficial or deeper strata of the epithelium. By contrast, in the dewaxed sections the reaction was more uniformly distributed throughout the epithelial layers (cf. figs. 4 and 5, Pl. 1). It is of interest that even though the superficial layers are keratinized in the guinea-pig, rat and mouse, some reaction was usually present in them. In the rat and mouse, some large cells (possibly macrophages) in the tunica propria gave a reaction, and in a few instances some reaction was present in a fibrous network lying between the fibres of the muscle coats.

In the dog's oesophagus, the submucosa contains a large number of lobulated glands, composed of mucous acini with serous crescents. The mucous portions gave a strong reaction, more especially towards the cell bases, but the serous crescents were negative. The ducts of these glands and their contained secretion gave no reaction (Pl. 1, fig. 6).

Gomori (1946) found that the oesophageal epithelium shows a uniform reaction throughout the deeper layers in all species he studied except in man, where it was patchy. Richterich (1952*b*), in a study of the mouse, found a reaction in only one oesophagus of several studied and it was present throughout the lining epithelial layers, though strongest in the stratum germinativum. A few places in the tunica propria also reacted.

The findings of Nachlas & Seligman (1949*b*) with the 'azo dye' technique correspond fairly closely with those reported here, namely, a reaction in the lining epithelial layers, increasing towards the basal layer, also in the tunica propria and muscularis mucosae of dog and rat and in the dog's oesophageal glands.

The stomach

In the rat and mouse, the proximal part of the stomach is lined, like the oesophagus, with keratinized stratified squamous epithelium, which forms a sharp junction with the fundus region proper; sections of the fundus in these species included the region of this junction.

(a) *The fundus region.* The rat alone gave no reaction in the stomach; even the stratified squamous epithelium was negative, which is strange since the similar epithelium in the oesophagus was in some specimens weakly positive. In the mouse, this epithelium was positive in some cases (Pl. 1, fig. 3).

Two patterns of reaction were encountered in the fundus region proper, the one exemplified by the mouse and guinea-pig, and the other by the dog, cat and rabbit.

In the mouse, the fundic glands near to the junction with the stratified squamous epithelium showed a reaction in the peptic cells, and these lie deeply in the glands. A weak reaction was given by the epithelial cells lining the gastric surface (Pl. 1, fig. 11). Distal to this short area near the junction region, the fundus was negative. These findings agree with Richterich's (1952*b*) observations. The guinea-pig gave an essentially similar result, though the reaction was somewhat weaker.

In the dog, cat and rabbit, there was no reaction in the glands, but in all three species the epithelial cells lining the ducts of the glands and the gastric surface were positive. The reaction was strong in the cat, and mostly confined to the ducts, where it occupied the same (supranuclear) part of the cell cytoplasm which gives a reaction for mucin: very little reaction was given by the surface epithelium (Pl. 1, fig. 7). In the rabbit, the reaction was of about the same degree of intensity in the epithelium of the gastric surface as in the gland ducts, and was not confined to a supranuclear position (Pl. 1, fig. 9). The reaction was weak in the dog, and was rather less in the surface epithelium than in the gland ducts (Pl. 1, fig. 10).

Additional sites of activity occasionally seen were large cells in the tunica propria, lying just below the epithelium in the dog and mouse, and what appeared to be a fibrous network between the cells of the outer muscle coat in the guinea-pig and mouse.

These results agree with Gomori's (1946) for the dog and rat, but are at variance with his negative findings for the rabbit and guinea-pig.

There are many points of similarity between the present findings and those of Nachlas & Seligman (1949*b*) and Chessick (1953) using the 'azo dye' technique, but there is not entire agreement. Results were similar for the mouse and rabbit (see Chessick) and dog (see Nachlas & Seligman). Although both authors reported the deeper parts of the gastric glands positive in the guinea-pig, a reaction in the surface epithelium was not recorded. Chessick's report of a reaction in the gastric glands of the cat and rat is at variance with the present findings.

A reaction in stellate cells and in fibres between the smooth muscle cells was noted in rat and mouse (Chessick) and a similar observation was made on some specimens of guinea-pig and mouse in the present work, but the smooth muscle reaction reported by Nachlas & Seligman for the dog and rabbit was not observed.

The nerve cells and fibres of the intrinsic ganglionated plexuses, reported positive by Nachlas & Seligman and also by Chessick, were found to be consistently negative with the 'Tween' technique.

The nature of gastric 'lipase'

Gomori (1948*a*), in a special study of mouse tissues with the 'Tween' technique, employed various activators and inhibitors that have been used by biochemists for distinguishing between true lipase and non-specific esterase. The most important of these are the bile salts (sodium taurocholate and sodium glycocholate), each of which activates lipase and inhibits non-specific esterase. Gomori found that bile salt, when added to the substrate bath, intensified the reaction in the pancreas and stomach, but inhibited that in other tissues. He later (1949) advanced further evidence that the enzyme of the stomach of certain species (man, monkey and mouse) is similar to that of the pancreas, since these were the only two tissues which hydrolysed unsaturated fatty acid esters at a fast rate. Although he gave no details of the actual localization of the enhanced gastric activity, he subsequently (1952) stated that true lipase is located in the pancreas, and also in the chief cells of the mouse stomach.

In the present investigation, the bile salt test was applied to stomach sections of the dog, cat, rabbit, guinea-pig and mouse, and it was found that in the dog, cat and rabbit, the reaction normally given by the mucous cells of the surface epithelium and gland ducts was markedly reduced or suppressed in the presence of bile salt (sodium glycocholate). In the guinea-pig and mouse, whilst the reaction in the mucous cells of the surface epithelium was suppressed, the reaction in the peptic cells was intensified.

The conclusion is drawn that the peptic cells of the guinea-pig and mouse contain true lipase, but that the mucus-secreting cells of the surface and gland duct epithelium of these and the other species studied contain non-specific esterase.

The pylorus

With the exception of the rat, whose pylorus was negative, all species gave a reaction (usually weak) in the ducts of the pyloric glands, but never in the glandular segments. Invariably the reaction was patchy, being present in some ducts and not in others. In the dog and cat, the reaction was confined to the supranuclear part of the cell, i.e. the portion which gives a reaction for mucin (Pl. 2, figs. 12, 13). There was scarcely any reaction in the surface epithelium.

In the rabbit, guinea-pig and mouse the reaction in the gland ducts was not confined to the supranuclear part of the cell, but was more generally distributed in the cytoplasm, and was often present in the surface epithelium as well.

Previous studies with the 'Tween' technique have demonstrated a reaction in the pylorus of only one of the above species. Gomori (1946) found that in the dog there was a reaction similar to that of the fundus, but the rabbit, guinea-pig and rat were negative. The mouse pylorus was reported negative by Richterich (1952*b*).

Again, the pylorus has shown little or no mucosal reaction with the 'azo dye' technique, though the nerve plexuses react (Nachlas & Seligman, 1949*b*).

The duodenum

Neither the cat nor the dog showed any reaction in the duodenum. All the other species gave a reaction in the epithelial cells lining the villi and upper parts of the crypts. This agrees with Gomori's (1946) observations for the rabbit, guinea-pig and

rat, and with Richterich's (1952*b*) for the mouse. The precipitate was normally scattered throughout the cell cytoplasm, but in some instances it was denser at the striated border and even appeared in the form of striations. This was particularly the case with the guinea-pig. Brunner's glands were found to be positive in the mouse, as Richterich (1952*b*) reported, but not in all specimens. No other species showed a reaction in the glands, but Gomori's (1946) observation that in the rabbit there are strongly positive cells among the non-reacting mucous acini, was confirmed (Pl. 2, fig. 14). These cells are serous in type and form discrete acini as well as crescents to the mucous acini, and they are of considerable interest in that they have long been believed to be of the same nature as those of the pancreatic acini. In a previous communication (Martin, 1954) support was given to this view. It was demonstrated that the enzyme in these cells is activated by bile salt, showing it to be a true lipase, and the pancreas is known to be one of the few tissues which contains true lipase.

The results obtained by Nachlas & Seligman (1949*b*) and Chessick (1953) with the 'azo dye' technique agree with the above findings for the villus epithelium, except that Chessick found a slight reaction in the cat. Their results differ however with respect to Brunner's glands, which they found to be positive, though of variable intensity, in all species studied. Chessick reported a positive network of cells and fibres between the smooth muscle fibres in the rat and mouse, but Nachlas & Seligman reported that a reaction in the muscle coats was slight, except in the dog. Both authors found the myenteric plexuses to be positive, as in other parts of the intestine.

The jejunum and ileum

The cat alone showed no reaction throughout the small intestine. In all the other species, the epithelial cells of the villi and upper parts of the crypts, but not the goblet cells, were positive as in the duodenum (Pl. 2, fig. 15). Often, the reaction in the ileum was more patchy than in the jejunum. It is noteworthy that although the dog showed no reaction in the duodenum, a patchy reaction was usually given by the remainder of the small intestine. This is contrary to Gomori's (1946) finding that the dog's jejunum as well as the duodenum was negative.

Apart from the lining epithelium, the only other sites of reaction were some large cells in the tunica propria of the dog (reported also by Gomori, 1946), and the smooth muscle coats of one mouse jejunum.

With the 'azo dye' technique, Chessick (1953) also found the cat's small intestine negative. Species which gave a reaction in the duodenal villi, i.e. rabbit, rat and mouse, also did so in other parts of the small intestine. Auerbach's plexus in all species, and the muscle coats of the rat and mouse, were positive. Nachlas & Seligman's (1949*b*) results do not agree with Chessick's, in that they found positive villi in the rat only.

The large intestine

In all species investigated the lining epithelium of the large intestine, with the exception of the goblet cells, was positive.

The pattern of the reaction given by the rabbit, guinea-pig and mouse was similar. In the caecum, a strong reaction was present in the epithelial cells lining the surface

and the upper parts of the crypts; towards the middle parts of the crypts, the reaction became less intense and finally disappeared in the deeper parts (Pl. 2, figs. 16, 17). Sections from the proximal and distal parts of the colon normally showed a less intense reaction, which extended for only a short distance into the crypts. In the dog and rat, a weak reaction was obtained in some specimens of the caecum, but there was little or none in the colon. In the cat, the reaction was uniform throughout the large intestine; it was present in the surface epithelium and extended into that of the crypts for not more than one-third of their depth (Pl. 2, fig. 18).

In addition to the reaction in the epithelium, some large unidentified cells in the tunica propria of the large intestine of the guinea-pig and mouse were strongly positive and there were occasionally some positive cells in the muscle coats of the mouse colon, lying between the smooth muscle fibres.

There is no previous report of a reaction having been found in the large intestine of these species by means of the 'Tween' technique. Gomori (1946) reported the large intestine of only man and monkey to be positive; in the latter species, the entire epithelium, including the goblet cells, reacted.

The 'azo dye' results of Nachlas & Seligman (1949*b*) are similar to those of the present study, but they did not study the caecum. The rabbit colon showed a supranuclear reaction in the surface epithelium. The colon of the rat gave an irregular reaction in the crypts and to a lesser extent in the tunica propria, whilst in the dog there was feeble activity in the crypts and some cells of the submucosa. As in other parts of the intestinal tract, the dog showed a reaction in the smooth muscle coats (muscularis mucosae and outer longitudinal coat) and in the nerve plexuses.

The salivary glands

The parotid gland is morphologically of serous type in the species investigated, but the nature of the submandibular gland varies from species to species.

It has previously been reported (Martin, 1953*b*) that the ducts of salivary glands give a reaction with the 'Tween' technique. This was the case in all species studied except the rat, whose salivary glands were completely negative. The duct system of the salivary glands was positive throughout in the cat, rabbit and mouse, but in the dog the reaction was found mainly in the large interlobular ducts (Pl. 2, fig. 19). The results for the guinea-pig were inconstant; some specimens showed no duct reaction whilst others did to a variable degree. No reaction was seen in duct contents, even when acini as well as ducts were positive.

A reaction in acini was less common than in ducts. Only the guinea-pig showed positive acini in the parotid gland (Pl. 2, fig. 21), although it should be mentioned that a few lobules of mucous acini were seen in one specimen of dog parotid, and these were positive.

Only in the dog and guinea-pig did the acini of the submandibular gland react. In the dog, the acini are mucous and have serous crescents: the mucous acini were strongly positive, especially towards the cell bases, but their crescents were negative (Pl. 2, fig. 20). The acini in the guinea-pig, which are of a type known as 'special' serous, were strongly positive. Neither the 'special' serous acini of the rabbit and mouse, nor the mucous acini (including their serous crescents) of the cat, gave any reaction.

Lobules of mucous acini of the sublingual gland were included with some of the submandibular gland sections of the guinea-pig and mouse, and these, as well as their ducts, were strongly positive.

Gomori (1946), who studied the salivary glands of the guinea-pig and rat, found no reaction in the ducts, but his observation that the mucous and serous acini of the rat are negative whilst those of the guinea-pig are positive, was confirmed. He noted some reacting cells lying outside the acini in the rat which he tentatively identified as basket cells: this feature was not observed in the present study.

Recently, workers using the 'azo dye' technique have also shown that the ducts of salivary glands are positive (Chessick, 1953; Hill & Bourne, 1954).

The reaction in fat cells

The observation of Gomori (1946) and others, that fat cells of some species show a reaction in their peripheral rim of cytoplasm, was confirmed. The fat cells were positive in four of the six species studied, i.e. rabbit, rat, mouse and cat. These species were also reported by Chessick (1953) to show positive fat cells with the 'azo dye' technique.

In some sections of mouse tissues, lobules of 'brown' fat were included, and their cells gave a reaction throughout the cell cytoplasm around the vacuoles from which fat had been dissolved out. This observation for the mouse was also made by Richterich (1952*b*) with the 'Tween' technique, and he concluded that the degree of intensity of the reaction is related to the functional state of the cell.

DISCUSSION

Of the sites of esterolytic activity described, but few can be attributed to the presence of true lipase. The pancreas is the main site, but in some species the gastric mucosa contains the enzyme in lesser amount. In following up some earlier biochemical observations of Volhard (1901), Willstätter & Memmen (1924) established for the pig that esterolytic enzymes extracted from the pancreas and the stomach show similarities in their biochemical behaviour, and that their activity is enhanced by either bile salts or sodium oleate. They found the stomach to be much weaker in activity than the pancreas, and that its activity was mainly located in the cardiac region. This has a parallel in the present histochemical observation that the peptic cells of the gastric glands near the cardiac region of the guinea-pig and mouse exhibit true lipase activity, whilst further distally they are negative. Other species studied showed no reaction in the peptic cells, but did so in the epithelium lining the gland ducts, where the reaction was found to be due to non-specific esterase.

In the present investigation, another site of true lipase activity was discovered, viz. the serous cells amongst the mucous acini of Brunner's glands in the rabbit, an observation which supports the view that they are of a similar nature to those of the pancreatic acini.

It is then, the serozymogenic cells which harbour true lipase. The serous type cells of very few sites give any reaction for 'lipase', i.e. they do not harbour even non-specific esterase. In fact, in this study the only positive serous type cells found were the acinar cells of the guinea-pig's parotid and submandibular glands. It is claimed

that the acinar cells of the guinea-pig's submandibular gland are serozymogenic, in contrast to those of most laboratory animals such as the rabbit, rat and mouse, which are (like the crescent cells of the dog and cat) of an ill-defined 'special' serous type (Stormont, 1932). However, it was found that the acini of the guinea-pig's submandibular gland give a weak periodic acid-Schiff reaction, which is not a characteristic of serozymogenic cells; they therefore appear to stand in a special class.

In contrast to the serous type cells, the mucous cells in many localities are 'lipase'-positive, and here the reaction is due to non-specific esterase. It is of interest that this reaction, as pointed out previously (Martin, 1953*b*), draws a distinction between cells of this class. In the first place, not all mucous cells of a particular species are positive, and secondly, the mucous cells of a particular region, such as a salivary gland, may be positive in one species, but not in others. Worthy of special mention is the fact that goblet cells in all species studied gave no reaction, but on the other hand, in all except the rat, the mucous cells lining the ducts of the fundic and pyloric glands, and to a lesser extent those of the general surface of the stomach as well, were positive: in the cat and the dog, precise localization to the supranuclear part of the cells (i.e. the mucus-secreting part) was clearly seen. This reaction in gastric gland ducts is a noteworthy feature in that in the majority of glands studied, the epithelium lining the ducts, even when not mucus-secreting, was positive, and often strongly so.

The functional significance of non-specific esterase in mucous cells and in gland ducts is not clear. The close spatial relationship of the enzyme reaction to mucus accumulation in a number of cells may indicate that it plays some part in the formation of mucin. There is no evidence from the histochemical findings that the enzyme is excreted from the cells; even when both mucous gland cells and the epithelium lining their ducts are strongly positive, no reaction is seen in the duct contents. This is in contrast to the true lipase of serozymogenic cells (e.g. of the pancreas), from which it is excreted and detectable histochemically in the duct contents. Its function is the hydrolysis of ingested fats.

The presence of non-specific esterase in the epithelium lining the ducts of glands would suggest that they have some physiological role apart from serving as conducting passages. Most investigations on the function of gland ducts have been made on the salivary glands. According to Babkin (1950), the larger ducts are properly regarded as purely conducting passages (except possibly in man), but the 'striated' intralobular ducts are known to undergo certain morphological changes when the gland passes from rest to activity, and they are thought to have a secretory function, the most likely one being the addition of water as a diluent of the secretion from the acini. However, if it can be assumed that the presence of non-specific esterase in the duct epithelium indicates some physiological role, it would mean that the larger ducts have more than a purely mechanical function, since they frequently give a stronger reaction than the intralobular segments.

In some species the lining epithelium of all parts of the alimentary canal is positive, but in others that of some parts is negative. This would suggest that non-specific esterase has a part to play in absorptive or excretory processes, but what this may be is not known. The enzyme is associated with different types of cells in the different parts of the canal, namely, the cells of the stratified squamous epi-

thelium in the oesophagus, the mucous cells of the surface epithelium and gland ducts in the stomach, and the columnar lining cells in the small and large intestine.

The reaction in the oesophageal epithelium was present in all species examined. It is difficult to explain its presence there, but it might be tentatively suggested that it is in some way concerned with the changes which the cells undergo in their migration to the surface.

With regard to the small intestine, it might be thought that here non-specific esterase would be concerned in fat absorption, since it hydrolyses fatty acid esters. There is no direct evidence, however, that this is the case, and a number of facts do not accord with this supposition. For example, the enzyme is not demonstrable in all sites where neutral fat is absorbed or stored, e.g. the duodenum of the dog, the whole of the cat's small intestine and the fat cells of certain species. In the dog's gall bladder, where stainable fat is present in the majority of the lining epithelial cells and is thought to be absorbed by them, the enzyme is present in relatively few of these cells.

The reaction in the large intestine, common to all species studied, and in some cases stronger than that given by the small intestine, is scarcely likely to be concerned in fat absorption, but it is difficult to provide an explanation for its presence. However, the fact that the large intestine gives such a strong reaction not only for alkaline phosphatase (Martin, 1951) but for non-specific esterase as well, would appear to indicate that this region may have a wider range of physiological activity than that commonly assigned to it, viz. the absorption of water and electrolytes.

There was no reaction in the intestinal contents, except in a few sections of duodenum, where it was probably due to lipase secreted from the pancreas. This accords with the observation of Connstein (1904) that the evidence is against the presence of a fat splitting enzyme in the intestinal juice itself, and also with that of Loevenhart (1902) that faeces show little activity towards ethyl butyrate.

With regard to the relation of the present results to those of other histochemical studies with the 'Tween' and 'azo dye' techniques, most of the results previously reported with the 'Tween' technique for the tissues studied here have been confirmed, but a number of new data are added. There is close agreement also with the results reported by those who used the 'azo dye' technique. However, certain sites that are consistently positive with this technique are negative with the 'Tween', namely, the intrinsic ganglionated nerve plexuses and the muscular layers of the intestinal wall. This difference is almost certainly accounted for by the fact that the 'azo dye' technique demonstrates sites of cholinesterase, as well as non-specific esterase activity. Histochemical study of cholinesterase distribution has shown that in the intestine, the ganglionated plexuses and muscle coats are almost the exclusive sites of activity (Gomori, 1948*b*). Again, nerve cells of the central nervous system and motor end plates react with both the 'azo dye' technique (Chessick, 1953) and the cholinesterase technique (Gomori, 1948*b*). Some of the other differences in enzyme localization which have become apparent between the 'Tween' and the 'azo dye' techniques may have another explanation. It may well be that a series of esterases exists in tissues, each with only a relative substrate specificity, and, though a wide variety of esters may be attacked by each of them, the use of different substrates would bring out differences in intensity of reaction in a par-

ticular site, or even some differences in localization. This view has already been put forward by workers who have used different substrates with the 'azo dye' technique (Chessick, 1953; Hill & Bourne, 1954).

SUMMARY

1. Using the 'Tween' technique, a detailed histochemical study has been made of the distribution of esterolytic activity (true lipase and non-specific esterase) in the alimentary tract and associated glands of laboratory animals (dog, cat, rabbit, guinea-pig, rat and mouse).

2. An improvement in the technique has been elaborated, and some new data have been obtained.

3. It has been found that, in addition to the pancreatic acini in all the animals, some of the peptic cells of the mouse and guinea-pig stomach, and the serous cells among the mucous acini in Brunner's glands of the rabbit, contain true lipase.

4. Mucus-secreting cells of many, but not all, sites are positive, as are also the cells lining the majority of gland ducts, whether mucus-secreting or not.

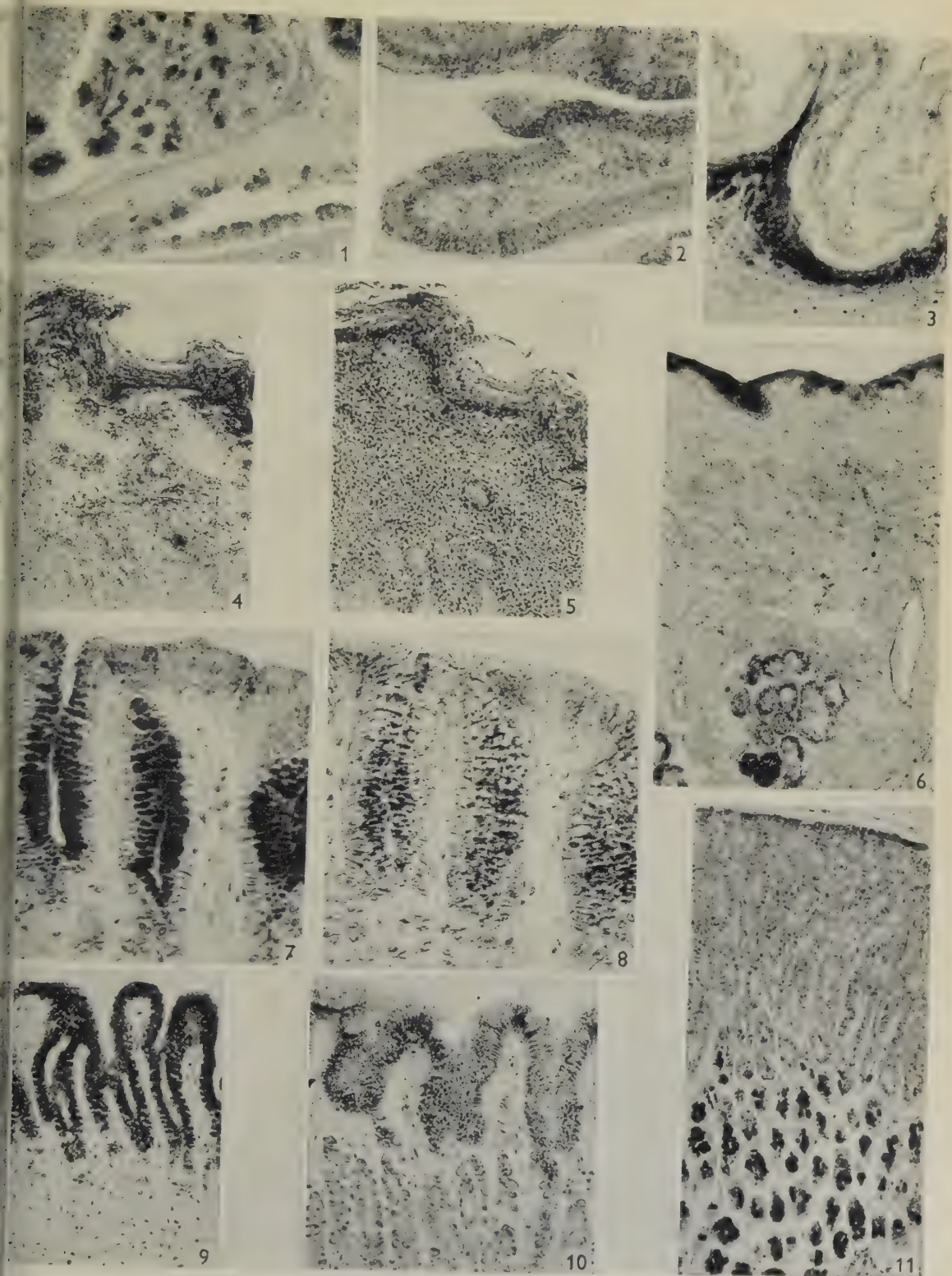
5. The lining epithelium of the large intestine, with the exception of the goblet cells, is positive, and often more strongly so than that of the small intestine.

6. A comparison is made between the results of the present study and those of other workers employing the 'Tween' and 'azo dye' techniques, and the relation of the present findings to some physiological problems is discussed.

The present investigation was carried out in the Department of Anatomy, University College, Cardiff. I should like to thank Dr F. Jacoby for his interest and encouragement, and Mr L. Jones for his assistance with the section cutting and photomicrography. I am indebted to Prof. Francis Davies for his advice on the preparation of the manuscript.

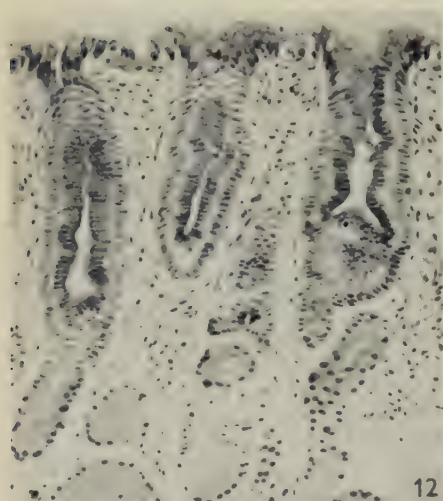
REFERENCES

- BABKIN, B. P. (1950). *Secretory Mechanism of the Digestive Glands*. New York: Paul B. Hoeber Inc.
- BARNETT, R. J. & SELIGMAN, A. M. (1951). Histochemical demonstration of esterases by production of indigo. *Science*, **114**, 579-582.
- BARNETT, R. J. (1952). The distribution of esterolytic activity in the tissues of the albino rat as demonstrated with indoxyl acetate. *Anat. Rec.* **114**, 577-599.
- CHESSICK, R. D. (1953). Histochemical study of the distribution of esterases. *J. Histochem. Cytochem.* **1**, 471-485.
- CONNSTEIN, W. (1904). Über fermentative Fettspaltung. *Ergebn. Physiol.* **3**, 194-232.
- GOMORI, G. (1945). The microtechnical demonstration of sites of lipase activity. *Proc. Soc. exp. Biol., N.Y.*, **58**, 362-364.
- GOMORI, G. (1946). Distribution of lipase in the tissues under normal and under pathologic conditions. *Arch. Path.* **41**, 121-129.
- GOMORI, G. (1948a). Histochemical differentiation between esterases. *Proc. Soc. exp. Biol., N.Y.*, **67**, 4-6.
- GOMORI, G. (1948b). Histochemical sites of choline esterase activity. *Proc. Soc. exp. Biol., N.Y.*, **68**, 354-358.
- GOMORI, G. (1949). Histochemical localization of true lipase. *Proc. Soc. exp. Biol., N.Y.*, **72**, 697-700.
- GOMORI, G. (1951). In *Methods in Medical Research*. **4**, sect. 1. Chicago: Yearbook Publ. Inc.
- GOMORI, G. (1952). *Microscopic Histochemistry. Principles and Practice*. University of Chicago Press.

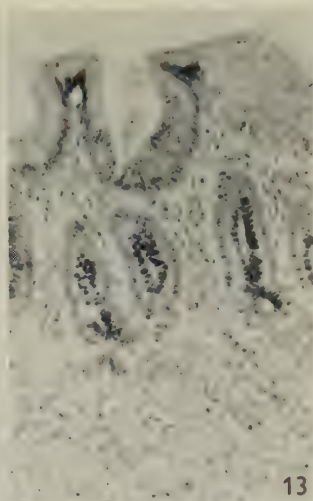


MARTIN—DISTRIBUTION OF 'LIPASE' IN THE ALIMENTARY TRACT

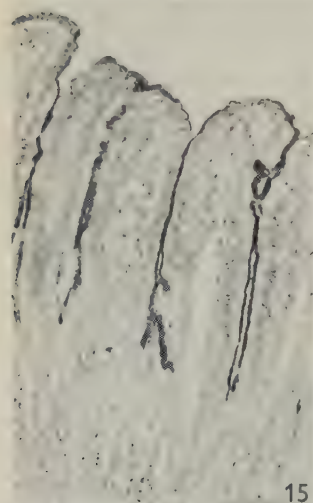
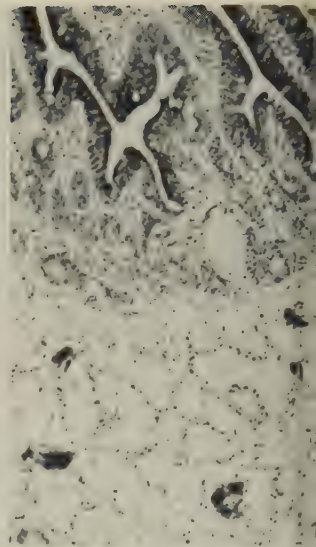
(Facing p. 452)



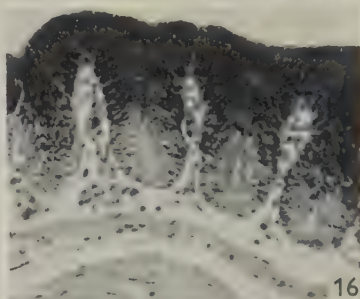
12



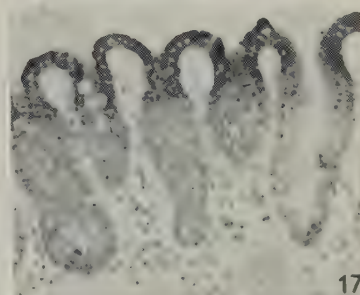
13



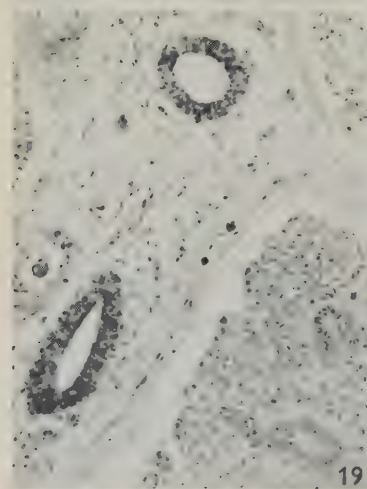
15



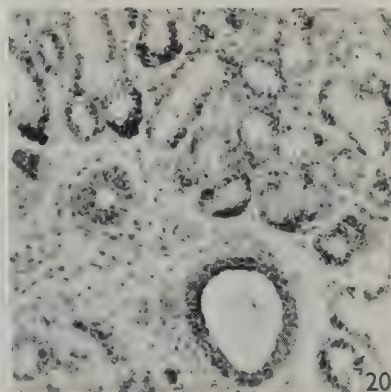
16



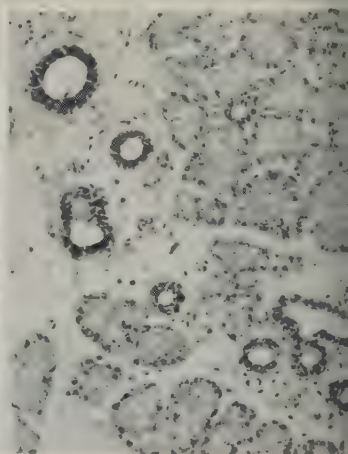
17



19



20



- HANRIOT, M. (1896). Sur un nouveau ferment du sang. *C.R. Soc. Biol., Paris*, **3**, 925-926.
- HILL, C. R. & BOURNE, G. H. (1954). The histochemistry and cytology of the salivary gland ducts. *Acta Anat.* **20**, 116-128.
- HOLT, S. J. (1952). A new principle for the localization of hydrolytic enzymes. *Nature, Lond.*, **169**, 271-274.
- HOLT, S. J. (1954). A new approach to the cytological localization of enzymes. *Proc. roy. Soc. B*, **142**, 160-169.
- LAGERLÖF, H. (1945). Normal esterases and pancreatic lipase in the blood. *Acta med. scand.* **120**, 407-436.
- LOEVENHART, A. S. (1902). On the relation of lipase to fat metabolism—lipogenesis. *Amer. J. Physiol.* **6**, 331-350.
- MARTIN, B. F. (1951). Alkaline phosphatase in the large intestine. *J. Anat., Lond.*, **85**, 140-158.
- MARTIN, B. F. (1953*a*). The histochemical test for lipase. *J. Physiol.* **119**, 24*P*.
- MARTIN, B. F. (1953*b*). 'Lipase' in gland duct epithelium and in mucus-secreting cells. *Nature, Lond.*, **172**, 1048.
- MARTIN, B. F. (1954). Serous cells in Brunner's glands of the rabbit. *Nature, Lond.*, **174**, 1195.
- MENTEN, M. L., JUNGE, J. & GREEN, M. H. (1944). Distribution of alkaline phosphatase in kidney following the use of histochemical azo dye test. *Proc. Soc. exp. Biol., N.Y.*, **57**, 82.
- NACHLAS, M. M. & SELIGMAN, A. M. (1949*a*). The histochemical demonstration of esterase. *J. nat. Cancer Inst.* **9**, 415-425.
- NACHLAS, M. M. & SELIGMAN, A. M. (1949*b*). The comparative distribution of esterase in the tissues of five mammals by a histochemical technique. *Anat. Rec.* **105**, 677-696.
- NACHLAS, M. M. & SELIGMAN, A. M. (1949*c*). Evidence for the specificity of esterase and lipase by the use of three chromogenic substrates. *J. biol. Chem.* **181**, 343-355.
- RICHTERICH, R. (1952*a*). Zur Technik des histochemischen Esterasennachweises. *Acta Anat.* **14**, 263-296.
- RICHTERICH, R. (1952*b*). Über die lokalisation einiger Esterasen in verschiedenen Organen der Albinomaus. *Acta Anat.* **14**, 342-352.
- RUYTER, J. H. C. & NEUMANN, H. (1949). A critical examination of the histochemical demonstration of the alkaline phosphomonoesterase. *Biochim. Biophys. Acta*, **3**, 125-135.
- STORMONT, D. L. (1932). The salivary glands. In Cowdry's *Special Cytology*, vol. 1, 2nd ed. New York: Paul B. Hoeber, Inc.
- VIRTANEN, A. I. & SUOMALAINEN, P. (1933). Untersuchungen über die Lipasen im Tierorganismus. (I Mitteilung). *Hoppe-Seyl. Z.* **219**, 1-21.
- VOLHARD, F. (1901). Ueber das Fettsplattende Ferment des Magens. *Z. klin. Med.* **43**, 397-419.
- WILLSTÄTTER, R. & MEMMEN, F. (1924). Vergleich von Magenlipase mit Pankreaslipase. *Hoppe-Seyl. Z.* **133**, 247-259.

EXPLANATION OF PLATES

Sites of 'lipase' activity appear black in the photomicrographs. Except where otherwise stated, all sections illustrated were incubated overnight (18-24 hr.) and were not dewaxed prior to incubation. Nuclei were counterstained with Harris's haematoxylin. All photographs were taken through a Wratten H filter.

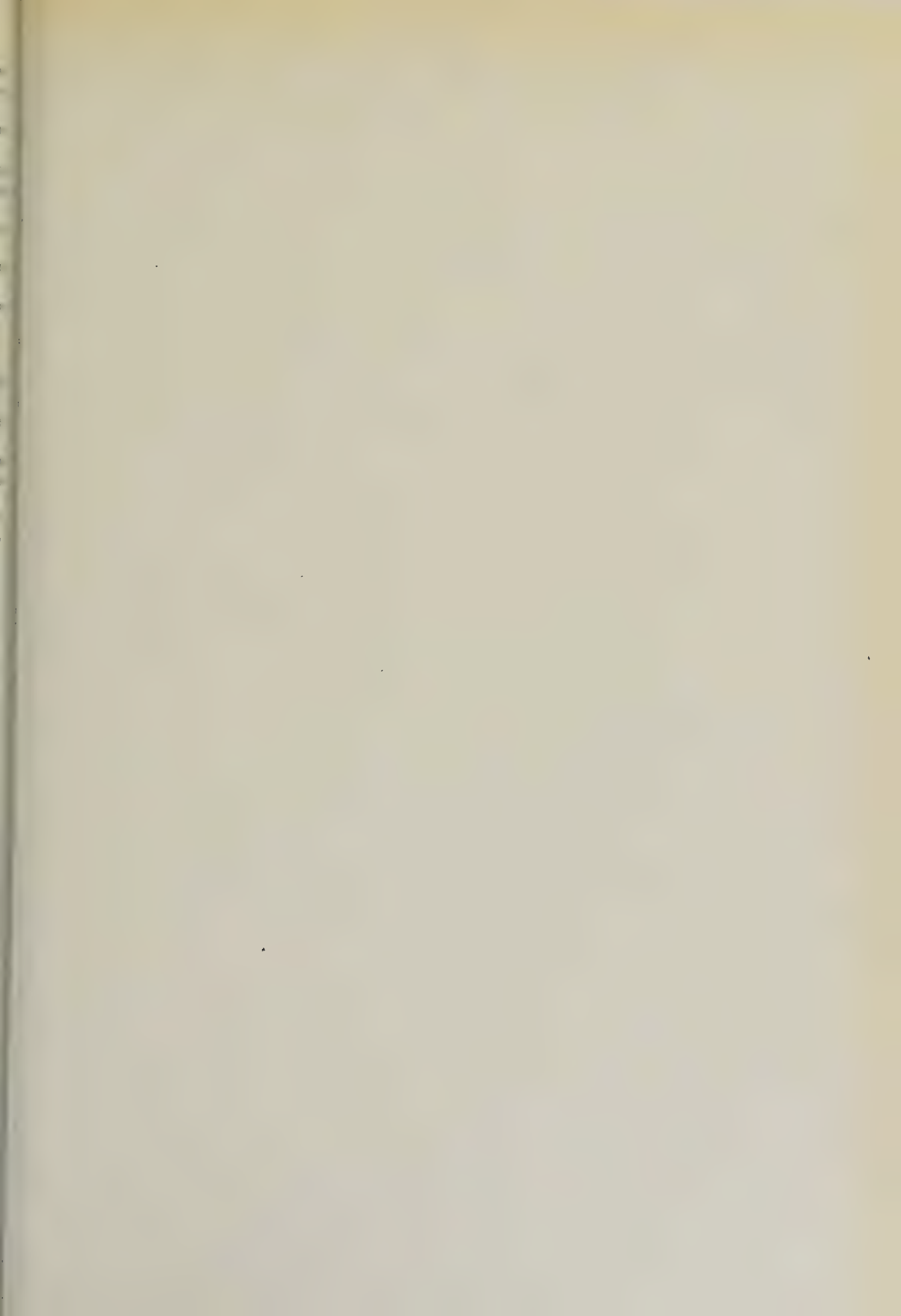
PLATE I

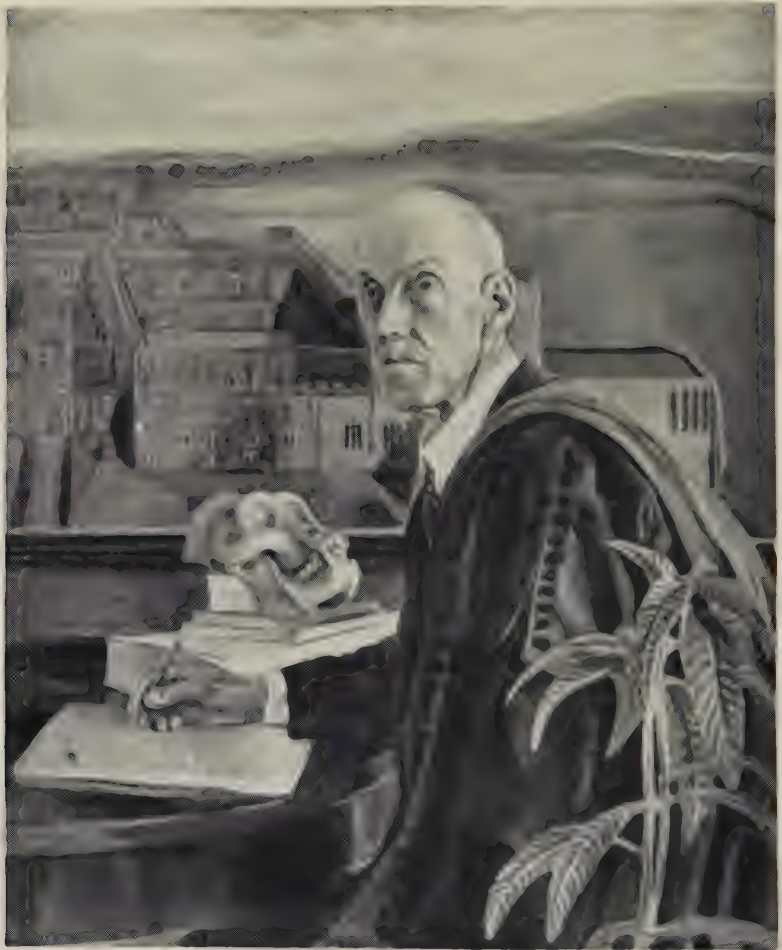
- Fig. 1. Cat, *pancreas*. A very intense reaction in the acini, especially in their central parts. A large interlobular duct shows a patchy reaction, and some positive contents are also seen. An islet seen to the left is negative. $\times 170$.
- Fig. 2. Dog, *gall bladder*. A patchy reaction in the lining epithelium. $\times 180$.
- Fig. 3. Mouse, *cardiac region of stomach*. A strong reaction in the lining epithelium (stratified squamous); similar to the reaction in the oesophagus. The superficial cornified layers are negative in this specimen. $\times 60$.
- Fig. 4. Guinea-pig, *oesophagus*. The strong reaction in the lining epithelium is patchy. $\times 120$.
- Fig. 5. Guinea-pig, *oesophagus*. This is the adjacent section to the one illustrated in fig. 4, but it was dewaxed prior to incubation. Note that the reaction is evenly distributed throughout the epithelium. $\times 120$.

- Fig. 6. Dog, *oesophagus*. In addition to a reaction in the lining epithelium, a strong reaction is given by the mucous oesophageal glands, especially towards their basal parts. The ducts of the glands and their contained secretion show no reaction. $\times 60$.
- Fig. 7. Cat, *fundus of stomach*. Strong supranuclear reaction in mucous cells lining the gland ducts, but little reaction in the surface epithelium. $\times 240$.
- Fig. 8. Cat, *fundus of stomach*. This is the adjacent section to the one illustrated in fig. 7, but it was dewaxed prior to incubation. Note the enormous reduction of the precipitate and poorer localization compared with fig. 7. $\times 240$.
- Fig. 9. Rabbit, *fundus of stomach*. Strong reaction in epithelium of surface and gland ducts; not confined to the supranuclear region of the cells. Glands are negative. $\times 130$.
- Fig. 10. Dog, *fundus of stomach*. Weak reaction in epithelium of surface and gland ducts. Glands are negative. $\times 100$.
- Fig. 11. Mouse, *fundus of stomach*. Strong reaction in peptic cells and slight reaction in surface epithelium. $\times 100$.

PLATE 2

- Fig. 12. Dog, *pylorus*. Supranuclear reaction in mucous cells lining the gland ducts, and a little reaction in surface epithelium. Glands are negative. $\times 160$.
- Fig. 13. Cat, *pylorus*. Supranuclear reaction in mucous cells lining the gland ducts. Glands are negative. $\times 130$.
- Fig. 14. Rabbit, *duodenum*. Strong reaction in villi, especially towards the striated border, and also in upper parts of crypts. Although Brunner's glands are negative, the serous acini and crescents among them are strongly positive. $\times 90$.
- Fig. 15. Guinea-pig, *jejunum*. Strong reaction in the lining epithelium of the villi, and almost confined to the striated border. Reaction passes for a short distance into the crypts. $\times 65$.
- Fig. 16. Guinea-pig, *caecum*. Strong reaction in surface epithelium and in approximately the upper half of the crypts. $\times 140$.
- Fig. 17. Mouse, *caecum*. Reaction in the surface epithelium and in approximately the upper $\frac{1}{3}$ of the crypts. $\times 145$.
- Fig. 18. Cat, *colon*. Strong reaction in surface epithelium and in approximately the upper $\frac{1}{3}$ of the crypts. Note negative goblet cells. $\times 115$.
- Fig. 19. Dog, *parotid gland*. Strong reaction in large interlobular ducts, and slight reaction in intralobular ducts. Serous acini negative. $\times 140$.
- Fig. 20. Dog, *submandibular gland*. Moderate reaction in interlobular ducts. Mucous acini positive, especially towards their basal parts. $\times 140$.
- Fig. 21. Guinea-pig, *parotid gland*. Strong reaction in the ducts, and weak reaction in the serous acini. $\times 130$.





DR WYNFRIED LAWRENCE HENRY DURRKORTH

IN MEMORIAM

DR WYNFRID LAWRENCE HENRY DUCKWORTH

On 14 February 1956, Dr Wynfrid Lawrence Henry Duckworth, Emeritus Reader in Human Anatomy at the University of Cambridge, died, having reached the age of 85 years.

Duckworth was the senior member of our Society. He was elected at a meeting held on 12 February 1899 at St Bartholomew's Hospital, when he was proposed by Alexander Macalister, then President, E. Barclay Smith and G. Elliot Smith, all of whom were closely connected with the University in which he lived and worked throughout his long and active life. In the Anatomical Society he was himself President for the years 1941 to 1943, and in 1950 was elected to Life Membership. Not only was Duckworth the senior member; he was also the last surviving member who had been elected during the nineteenth century.

Perhaps the most striking feature of Duckworth's career was his life-long devotion to his University, to Jesus College, to the Cambridge Department of Anatomy and to its collection of anthropological and primate material. He entered Jesus College as a Scholar in 1889, and four years later, having obtained a 'double first' in the Natural Sciences Tripos, became a Fellow of the College and a University Demonstrator in Anatomy. He at once became interested in physical anthropology and in primate anatomy, was appointed Lecturer in this subject in 1899, and made numerous communications to the Anatomical Society, many of which were published in this *Journal* and elsewhere. Collected together they made a volume of some 300 pages, published as *Studies from the Anthropological Laboratory, the Anatomy School, Cambridge*, in 1904, the same year which saw the publication of his principal work *Morphology and Anthropology, a Handbook for Students*. At the time, this was the only attempt at a comprehensive account of the Primates, from the point of view of an anatomist, available in English; it remained for many years a standard work and was a considerable achievement, completed at the relatively early age of 34 years. It still remains an important source of information in its field.

It is less well known that in 1907 he published an important paper on the histology of the placenta of *Macacus* in a very early implantation stage, and this at a time when our knowledge of early primate embryology was very scanty. In 1912 his only publication which could be called popular appeared in the form of a small volume on *Prehistoric Man* in the Cambridge Manuals of Science and Literature. It was a model of its kind, summarizing admirably the knowledge then available, and avoiding much of the premature speculation which is often a feature of such literature. It was perhaps inevitable as College business and administration came to occupy more of his time (he was Master of his College from 1940 to 1945) that Duckworth's scientific publications became less frequent as the years passed, but his anatomical interests were always maintained. In 1947, when he had reached his 77th year, he gave the Huxley Memorial Lecture on 'Some Complexities of Human Structure' before the Royal Anthropological Institute of Great Britain and Ireland;

in the following year, drawing on an extensive and scholarly knowledge of the history of his subject, he gave the Linacre Lecture at St John's College, on 'Some notes on Galen's Anatomy'. This appeared subsequently as a pamphlet, and was indeed his last publication.

Duckworth's position as one of those distinguished British anatomists who have contributed so much to our knowledge of primate anatomy and physical anthropology is assured. He is less well known than some, mainly because his interests did not lie predominantly in the fields of theory and speculation, and he took little active part in the many controversies which caused so much heat and not a little personal ill-feeling during the period in which he lived. He had a deep respect, one might almost say a love, for the actual specimen or dissection for its own sake, and a reluctance to speculate where he felt the evidence from observation was inadequate, or to endorse one interpretation when others appeared equally probable. It is noteworthy that he did not accept the evidence of the Piltown fragments without reservations, which, as he wrote in a characteristic sentence, 'constitute a safeguard... against the danger of allowing the intensive study of one specimen to bias judgement, and against the needless reiteration of old arguments in the absence of new evidence'. It might be said that in his work he was too closely bound to the material specimen, and particularly to the specimens in the Museum of the Department of Anatomy in Cambridge. This gave to his writings a somewhat disconnected character; observations remained isolated without the benefit of theory to draw them together into a single picture. At the same time it gave an actuality and a reliability to his work which is lacking in much which has been written on the subjects in which he was mainly interested. In any branch of science there is room for work of many different kinds, and Duckworth's will have a permanent and honourable place in the history of our knowledge of the Primates and of human evolution.

While his work will remain his most permanent memorial, it is as a teacher and a friend that Duckworth will chiefly be remembered by many generations of Cambridge medical graduates. He was indeed a characteristic Cambridge figure, with the dignity, courtesy and humour which, one likes to think, is to some extent a product of that environment. His friendly presence will be greatly missed in the University, in the College and in the Department where he has been known for so long. It is trite, but none the less true, to say that those places will never seem quite the same to those who knew him there.

F. GOLDBY

[The plate accompanying this *In memoriam* notice was made from a photograph of the portrait of Dr Duckworth in the possession of Jesus College, Cambridge. The portrait was painted by Mr James Wood in 1943. It is reproduced here by the courtesy of the Master and Fellows of the College.]

REVIEWS

Cardiovascular Innervation. By G. A. G. MITCHELL. (Pp. xii+356; 202 figures; 2 tables; $9\frac{1}{2} \times 6\frac{1}{2}$ in.; 55s.) Edinburgh and London: E. and S. Livingstone Ltd. 1956.

In 1953, Prof. G. A. G. Mitchell, whose researches on the autonomic nervous system over many years are well recognized, wrote an excellent monograph on the *Anatomy of the Autonomic Nervous System*. In that book the innervation of the heart and blood vessels was not treated in an exhaustive fashion and the present monograph is intended to meet that need. There is, however, a very considerable overlap in the two works and much matter described in the first book is repeated in the second. For instance, chapters I-VI (pp. 1-159) of the monograph on *Cardiovascular Innervation*, which take up approximately one-half of this book, deal with general principles, autonomic representation in the cerebrum, cerebellum, brain stem and spinal cord, autonomic outflows, endings, efferents and afferents, and a general consideration of the peripheral parts of the autonomic system. These subjects are covered, in general in somewhat more detail, in the earlier work, though some of them are considered in more detail in the present monograph, and the results of more recent researches are incorporated and some new matter is introduced. The remaining chapters VII-X (pp. 160-309) deal in detail with autonomic vascular innervation on a regional basis and provide an excellent survey of knowledge pertaining to this subject. Approximately one-half of the illustrations in this volume are the same as those in the earlier one; in both books the two tables are the same and there is necessarily very considerable repetition in the extensive literature cited. These observations are not made in the sense of adverse criticism of the present monograph, though some may maintain that a single monograph embracing the material in both these books would be of more value as a work of reference.

Considered on its own, however, the monograph on *Cardiovascular Innervation* is to be highly commended and will undoubtedly be a standard book of reference. The author has incorporated the results of many original studies made by himself and his school. The illustrations are excellent and very well reproduced; they include many photomicrographs of material prepared by the author. Anyone who has tried, by various techniques, to demonstrate the finest peripheral nerve plexuses and terminations will appreciate the difficulties and uncertainties of the task. In this book there are a number of striking illustrations of extremely fine terminal nerve networks, revealed by a combined intravital and supravital methylene-blue technique which has given excellent results in the hands of the author and his colleagues. No magnifications are recorded for about one-third of the photomicrographs. The work is primarily anatomical and concerned with the disposition of the autonomic nervous system in man, though information gleaned from embryological, experimental, pathological and clinical sources is freely used where necessary to explain or illumine certain features. So far as the reviewer is aware, no relevant contribution of major significance has been omitted from the voluminous literature quoted, and it forms a very valuable feature of the work. The book is attractively produced and considering the large number of illustrations, many of which are coloured, it is reasonably priced. The author is to be congratulated for writing a splendid monograph that will be widely appreciated.

F. DAVIES

The Anatomy of the Rat. By EUNICE CHACE GREENE. (Pp. xi+370; 339 figures; \$25.00 or £9.) New York: Hafner Publishing Co. 1955.

The laboratory rat is so important biologically that the reappearance of the most extensive monograph devoted to its anatomy is likely to be welcomed by such workers and institutions as can afford the very large sum which it now costs. The present issue is, apart from the cor-

rection of some errata, a facsimile of the original 1935 edition. A few minor typographical slips remain: for instance, on pp. 86, 87 and 118 the words 'Simiidae', 'Rivinian' and 'parotid' are incorrectly spelt; on p. 5 the zygomatics are omitted from the list of human skull bones; and in the table of carpal elements on p. 8, carpal I is included in the proximal row.

As an atlas, this book has much to commend it. The figures, in which the blood vessels and nerves are coloured, are clear and accurate, and provide, in spite of occasional excesses of detail, a concise visual record of actual dissections. In practice they certainly lighten the burden of identification, and form, therefore, a useful standby for experimentalists and others who have to find their way about the rat. Few, however, will share the author's concern for the extreme minutiae of peripheral nervous and vascular patterns. The topographical descriptions are intended only as an adjunct to the figures. As such they are usually adequate, although one might cavil at some statements, e.g. that the os penis lies on the ventral wall of the organ (it is dorsal to the urethra, in the sense that the dorsal artery and vein of the penis are dorsal), and that there are two 'ossa' (*sic*) uteri (the primitive uterus duplex is modified in the rat). Moreover, it is a pity that many structures should have been treated with a brevity which belies their importance. No more than five lines are given to the brain, which even in the figures appears only in external view, four lines to the spinal cord and two lines to the teeth. The heart, too, is poorly figured, and is not described in the text at all. There is no account of the levator ani—a muscle which has received attention recently in determinations of the myotrophic action of steroids.

Apart from its undoubted value as a dissection guide, the introduction and preface to the book both invite for it further consideration, as a study in comparative anatomy. This claim must be accepted with reserve. Partly as a result of the total exclusion of histology, the animal is described with almost no regard to function. Thus, although a good account is given of the general appearance and situation of the salivary glands, this does not include the secretory pathways, and there is no indication which glands are of serous and which of mucous type. The only basis of comparison throughout is the text-book anatomy of man: in consequence, the work has a grossly anthropocentric bias. Neither the similarities between human and rodent anatomy, which have so impressed the author, nor the dissimilarities—whether or not related, as she suggests, to differences in posture—can be truly assessed without reference to other mammals. So we find the straight head of the rectus femoris muscle and the inferior ventral iliac spine described with no suggestion that they are better developed in the rat than in many mammalian forms apart from man. The scraps of general morphology which are introduced are often archaic and dubious. The nodules of secondary cartilage at the ends of the clavicle are interpreted, without comment, as vestiges of the procoracoid and omosternum. In the tarsus, the whole talus is regarded as an os intermedium, the sesamoid element associated with tibialis posterior being the os tibiale. In spite of the prominence given to the vascular system, no one would guess that one of the features in this, as in that of other relatively primitive mammals, of greatest interest to comparative anatomists and embryologists is the persistence of a functional stapedial artery. Perhaps, after these strictures, it would be captious to add that the animal in question is now commonly placed in the genus *Rattus* rather than *Mus*.

All things considered, this re-issue represents an opportunity lost to produce a full and balanced study of a mammal which is interesting alike for its specializations, for the retention of primitive anatomical characters, and as a vehicle for medical research.

C. C. D. SHUTE

Proctologic Anatomy. By R. V. GORSCH. (Pp. xvi+310; 155 illustrations and 8 plates; $9 \times 5\frac{3}{4}$ in.; 61s. 6d.) 2nd ed., London: Baillière, Tindall and Cox Ltd. 1955.

In his preface to this book, which first appeared in 1941 as *Perineopelvic Anatomy*, the author states that he has aimed at bridging the gap between anatomist and practising proctologist. Certainly there are few parts of the body where such a bridge between the dissecting room and the operating theatre is more needed than in the pelvis, and anatomists will find much of value in Dr Gorsch's monograph.

Written by a surgeon, it is essentially a text-book of anatomy in which the practical application of the features described is indicated with commendable discrimination. Separate chapters, each with a good bibliography, deal with such topics as the anal canal, ano-rectal musculature, perineopelvic spaces, pelvic fascia, etc., and inevitably with such a treatment there is a good deal of necessary repetition. The illustrations, which are well chosen, include a series of photographs of the author's own dissections, but praiseworthy as this is, it is doubtful if the result is really successful.

In certain instances the terminology differs from current British usage, although the meaning is seldom in doubt; eponymous terms of long standing make (for the reviewer) a welcome reappearance, but unfortunately they have been joined by a multitude of more recent eponyms which seem hardly necessary.

The pelvic fascia is described at great length, and presumably the detail which is entered into possesses the merit of proven usefulness in surgical application. Surgeons of experience may find the account lucid enough, but to other than pelvic specialists it may prove rather heavy going. The chapter devoted to the perineopelvic spaces must also be considered surgical rather than anatomical. Several of the spaces described are merely planes of cleavage determined by pathological processes, but to the operating surgeon the account given should prove most helpful.

The brief statement on p. 219 referring to the course of the dorsal vein and nerve of the penis is misleading, and the description on p. 223 of the iliac fascia splitting into three layers is unusual. In the account of the anal canal its upper limit is taken as the dentate (pectinate) line, a view which is at variance with the higher limit accepted in this country. With regard to its lining epithelium the use of the term 'transitional' seems undesirable, and the statement that normally only traces of mucous membrane are found in the canal—even if the author's limits of extent are accepted—wrongly restricts the definition of such a membrane to one actually containing mucus secreting cells. Papers on the anatomy of the anal canal which have appeared only very recently, and which could not be considered by the author, will probably lead to some modification of this chapter in future editions.

There can be few matters relating to the anatomy of the lower bowel and pelvic floor upon which proctologists will not find valuable information in this book, and it can be confidently asserted that it will find a place on their shelves. The format is pleasing and typographical errors are few.

E. W. WALLS

Radio-Anatomie Générale de la Tête. By R. ANBANIAC and J. POROT. (Pp. 152.) Masson et Cie. 1955.

This work, written by an anatomist and a radiologist, is essentially a cross-sectional atlas. The authors cut frozen sections of the head and neck in three planes, frontal (coronal), sagittal and horizontal, at centimetre intervals. There are sixteen frontal, sixteen horizontal and six sagittal sections. The last consist of a mid-line cut and five cuts at centimetre intervals as far out as the lateral border of the orbit. The sections are well drawn (in black and white) to life size. Each has been radiographed and a radiographic print (again life size) accompanies each drawing. All are loose leaf.

The structures best displayed on the radiographs are the bones and brain, but the muscle sections are fairly well seen. The blood vessels and nerves cannot be made out. The radiographs are virtually tomographs or pneumotomographs, for the C.S.F. is naturally replaced

by air shadows which stand out well in negative contrast. The authors might well have made more of this, as besides the ventricles all the cisterns are very well displayed. Unfortunately the brain is incompletely labelled in this respect so that the student must work these points out for himself. It is well known that the cerebral ventricles collapse in death so they appear smaller than would be the case in tomograms on the living subject with air in his brain.

The authors are to be congratulated on presenting both anatomy and radiology in a new fashion. Being loose leaf the atlas is somewhat difficult to handle and can only be read on a large surface area. The seventy-six pictures each measuring 9 by 6 in., and rather more with the legends, could well be displayed behind glass on the wall of either the dissecting rooms or the X-ray department.

J. W. D. BULL

BOOKS RECEIVED

Histologie und mikroskopische Anatomie des Menschen mit Berücksichtigung der Histophysiologie und der mikroskopischen Diagnostik. By O. BUCHER, 2, neu-bearbeitete Auflage, 1956. (Pp. 571; 381 illustrations; Ganzleinen. Fr./D.M. 68.) Bern and Stuttgart: Medizinischer Verlag Hans Huber.

Histologie und mikroskopische Anatomie des Menschen. By W. BARGMANN, 2, verbesserte Auflage, 1956. (Pp. xvi + 796; 640 illustrations; Ganzleinen. D.M. 69.60.) Stuttgart: Georg Thieme Verlag.

Recherches sur la constitution de l'os adulte. By J. VINCENT. (Pp. 112; 41 illustrations.) Bruxelles: Editions Arscia.

Letalfaktoren in ihrer Bedeutung für Erbpathologie und Genphysiologie der Entwicklung. By E. HADORN. (Pp. 338; 129 illustrations; Ganzleinen. D.M. 39.) Stuttgart: Georg Thieme Verlag.

THE DEVELOPMENT OF THE PENILE URETHRA IN THE PIG

BY T. W. GLENISTER

Charing Cross Hospital Medical School

The mode of development of the penile urethra of the pig has been studied in order to discover whether the pattern of development in this animal is different from that described in man (Glenister, 1954). Ungulates are of special interest in this respect, as their penis becomes incorporated in the abdominal wall in the course of development.

LITERATURE

Although some of the earliest work on the development of the phallic region was carried out on human material by Tiedemann (1813), Meckel (1815) and Müller (1830), embryos from various species of ungulates have been used by several embryologists for the investigation of the development of this region. This was so presumably because this kind of material was available in abundance at a time when human embryos were difficult to obtain.

The three above-mentioned authors affirmed that the penile urethra was formed by the closure of a urethral gutter that extended along the under-surface of the penis. Rathke (1832) seems to have been the first author to become interested in the comparative aspects of the subject, and he stated that the closure of the urethral gutter to form the penile urethra starts at points which differ from mammal to mammal. Thus in rats the closure starts at the perineum, whereas in most ruminants it begins at the anterior extremity of the genital tubercle; in the pig the middle portion of the gutter is the last to close.

These classic descriptions were restated by Valentin (1835), Bischoff (1842), Koelliker (1861) and Cadiat (1884). The latter used mainly sheep embryos for his investigation, but also examined a limited number of pig and human embryos. He pointed out that the groove on the under-surface of the glans clitoridis and the vestibular region in the female correspond to the penile portion of the urethra in the male. Tourneux (1888), Fleischmann (1902-7), Schwartztrauber (1904), Böhm (1905) and Durbeck (1907) described the development of the external genitalia in sheep, pig, cat, guinea-pig and mole. These five authors state that the genital tubercle contains a low epithelial lamina (*lamina urethralis*), which protrudes into the mesoderm of the tubercle and is continuous caudally with the cloacal plug (*lamina cloacalis*). They do not state clearly what the role of the lamina urethralis is in the development of this region, but claim that the urorectal septum forms the perineum and the perineal raphe, and is thus responsible for the closure of the urethral groove. Thus, according to these authors, the urethral groove is not closed by the fusion in the midline of laterally placed urethral folds. The cranial displacement of the tip of the penis up to the umbilicus is explained by assuming that, in

male embryos, the perineum grows rapidly and is lengthened in a cranial direction to be incorporated in the abdominal wall.

Retterer (1890-1915) investigated the development of the urogenital system in horse, pig and sheep, as well as man, many rodents and many carnivores. His writings are so full of invective against Fleischmann and his school that it is often difficult to follow his arguments. He does, however, state quite clearly that the penile urethra results from the fusion of urethral folds, starting at the base of the organ and proceeding to the tip, this fusion giving rise to the perineal raphe. The urethral plate results from the outgrowth of urethral folds on either side of it and is definitely ectodermal in origin. Retterer does not, however, state clearly what part, if any, it plays in the formation of the urethra. This organ is, however, stated to be lined by epithelium derived from ectoderm and is formed throughout by the fusion of urethral folds. Retterer pours scorn on the concept presented by Wood Jones (1910) according to which the terminal urethra is derived from a separate ingrowth of ectodermal epithelium. It is, however, to Retterer's credit that he was not in the least impressed by the opinions of Felix (1912) which have so long formed the basis of accounts of the development of this region.

Retterer explained the close relation of the penile tip to the umbilicus in ungulates by describing a thickening of the abdominal wall extending from the linea alba to the penis. This thickening extends as a sling to the base of the free portion of the developing penis and so fixes it close to the umbilicus. Later a glandopreputial lamella grows in, separating the penis from the surrounding abdominal wall tissue, to give rise to an extensive preputial space. The free margin of the prepuce grows forward in the form of a preputial fold which gradually covers the whole of the free portion of the penis.

In 1924 Zietschmann stated that the cranial migration of the phallus is caused by the lengthening of the axial parts of the phallus underneath the perineal skin, the male genital tubercle of ungulates never developing into a cylinder which is free on all sides as in man. The enclosed main portion develops further, attached by means of connective tissue to the ventral abdominal wall.

In 1945, however, Broman compared the development of the penis in Primates with its development in Artiodactyla, and concluded that the penis which is enclosed in the abdominal wall is always developed ontogenetically from a *penis liber*, while the definitive penis liber never has a preliminary stage of being a *penis appositus*.

In 1947 the same author published a detailed account of the origin and secondary displacement of the external genitalia in ruminants, with special reference to the recognition of sex in pig, cow, sheep, roe deer, red deer and elk. This author describes a fusion of the urethral folds to form the penile urethra, and also describes a penile basal ring that anchors the base of the organ to the caudal part of the abdominal wall which in turn is drawn up towards the umbilicus. Thus the free portion of the penis is drawn cranially, the portion enclosed in the abdominal wall is greatly lengthened and the part of the penis that was at first free is completely drawn into the abdominal wall. Later this penile basal ring takes part in the formation of the definitive prepuce. Broman confined himself to the macroscopical appearances of the external genitalia and expressed no opinion about the origin and development of the urethral plate and urethra.

MATERIALS AND METHOD

The caudal parts of a series of thirteen pig foetuses ranging in size from 11 to 85 mm. crown-rump length, and the penis and abdominal wall of a pig foetus of 125 mm. crown-rump length, have been sectioned serially either in the coronal, the sagittal or the transverse plane. All specimens were measured after fixation, and were selected because the external genitalia presented either indifferent or male characteristics according to the criteria of Broman (1947). The sex of the foetuses was checked by means of the histological appearances of their respective gonads, the criteria of Gillman (1948) being applied.

DESCRIPTION OF MATERIAL

At 11 mm. the genital tubercle is barely identifiable with the naked eye and there are no genital swellings; the gonads are at the indifferent stage of differentiation.

The cloaca is an undivided cavity separated from the surface of the embryo by a thick cellular plate—the cloacal membrane. The urethral plate consists of a short cellular projection extending from the anterior wall of the cloaca and extending into the base of the phallic tubercle (Pl. 1, fig. 1). The constituent cells appear to be derived from the internal cloacal wall and to be distinct from the surface epithelium.

It should be noted that two sheep embryos of 13 and 15 mm. crown-rump length respectively were also examined, and showed quite clearly that the urethral plate consists of cells which are continuous with and indistinguishable, under the microscope, from those lining the internal cloacal or future urogenital sinus wall. In these two specimens the urethral plate is also quite distinct from the surface epithelium.

At 16 mm. the phallic tubercle of the pig embryo is in the form of a cone on to which ill-defined urethral folds extend from the margins of the ectodermal cloaca. The tubercle measured 1 mm. and no well-defined genital swellings were seen. The gonads are still at the indifferent stage of differentiation. The urethral plate extends along the under-surface and towards the tip of the phallic tubercle as a cellular proliferation from the anterior wall of the endodermal cloaca and urogenital sinus.

At 18 mm. the phallic tubercle is more elongated and measures 1.4 mm. The urethral folds are more clearly demarcated than in the 16 mm. specimen. No genital swellings have been observed and the gonad is still in the indifferent phase of differentiation. The urethral plate consists of a lamella extending into the phallus from, and derived from, the fused anterior portions of the walls of the urogenital sinus. The lower margin of the plate is in contact with surface epithelium which is two cells thick and which forms the roof of the ill-defined urethral groove. In the region of the tip of the phallus, the urethral plate extends to the dorsal surface of the tubercle. Where the urethral plate comes in contact with surface epithelium the plate tends to be thickened. The surface epithelium shows no sign of proliferation.

At 20 mm. the phallus is 1.5 mm. long and consists of a rounded knob situated

on a conical elevation. The urethral folds and groove extend on to the under-surface of both, and the tip of the knob is marked by a pit. Vague hillocks flanking the base of the phallic cone represent the primordia of the scrotal swellings. The gonads are early testes with well-defined sex cords and spindle-shaped cells under the coelomic epithelium forming the tunica albuginea.

The urogenital sinus and the rectum are completely separated, and the urethral plate extends into the phallus from the fused walls of the sinus. The dimple near the tip of the phallus indicates the site of an ingrowth of surface epithelium to meet the most anterior part of the urethral plate.

At 22 mm. the phallus measures 2 mm. A pit is again noted at the tip of the glans (Pl. 1, fig. 2), which is well defined in this specimen; the pit is in relation to the anterior extremity of the urethral groove. The genital swellings are poorly defined and situated on either side of the base of the phallus. The gonads can again be observed to be developing testes.

The definitive urogenital sinus is clearly divided into an antero-posteriorly compressed pars pelvina and a laterally compressed pars phallica. The urethral plate is formed from the fused walls of the urogenital sinus and is completely lamellar in form except for the deepest, most dorsal portion which is slightly thickened. The phallic portion of the urogenital sinus extends some way into this thickened part of the plate. This lamella lies in the roof of the groove produced by the bulging urethral folds (Pl. 1, fig. 3), and the surface epithelium in relation to the inferior margin of the plate is 'destratified' and appears to be retrogressing. Near the tip of the glans, the urethral plate forms a lamella occupying about two-thirds of the depth of the phallus. The urethral plate is quite distinct from the surface epithelium which is folded-in to meet the lower margin of the plate. These appearances probably result from the outgrowth of urethral folds on either side of the urethral plate. Sections through the tip of the phallus show that the terminal pit, already noted in the description of the external genitalia, is the site of an ingrowth of surface epithelium which meets the distal extremity of the endodermal urethral plate (Pl. 1, fig. 4).

At 25 mm. the phallus is curved caudalwards and is 2.5 mm. long. The primitive urogenital ostium is visible at the base of the phallus and is continuous with the urethral groove which is deeper in its proximal portion. The groove becomes progressively shallower as it is traced towards the tip of the phallus which is surmounted by a globular glans. The urethral folds flank the primitive urogenital ostium and the urethral groove, which is continued on to the under-surface of the glans, whence it may be traced to a definite pit near the summit of the organ. There is no vestige of an epithelial tag. The scrotal swellings are now quite evident and are situated in relation to the cranial part of the base of the phallus.

At 27 mm. the external genitalia present the same features except that the phallus is more markedly curved, so that the distal half is set at right angles to the proximal half (Pl. 1, fig. 5).

As these two stages present such similar appearances it is convenient to describe their histological features together.

The gonads are early testes and the pelvic and phallic portions of the urogenital sinus are clearly defined in both foetuses. The lumen of the phallic portion opens

out on to the surface at the primitive urogenital ostium, and this in turn is continuous with the urethral groove (Pl. 1, fig. 8). The part of the urethral groove immediately in front of the ostium is formed by the outgrowth of urethral folds lined by surface epithelium as in the previous cases, but it is deepened by the disintegration of the lower (ventral) thickened part of the urethral plate. The site of the latter is still indicated by epithelial bridges (Pl. 1, fig. 7). Farther forward along the phallus, the urethral groove is shallower (Pl. 1, fig. 6), the urethral plate is lamellar in outline and the surface epithelium, lining the roof of this part of the groove and in relation to the lower margin of the plate, gives the appearance of retrogressing, being 'destratified' and stretched out. As sections are traced towards the tip of the phallus, it is found that the epithelium lining the urethral groove is composed of as many layers of cells as are found in the epithelium lining the other surfaces of this region of the phallus. At the tip, an ingrowth of cells derived from the surface epithelium meets the distal part of the urethral plate. It has not been possible to determine in these specimens whether there is an additional ingrowth of surface cells from the terminal part of the urethral groove to meet the terminal part of the urethral plate, or whether the apparent infolding of surface epithelium is due to the outgrowth of the urethral folds.

At 32 mm. the phallus is 2.5 mm. long and has a distinct curvature in a caudal direction (Pl. 1, fig. 11), but it is not so marked as in the 27 mm. specimen. The distal limit of the urogenital ostium has extended to half-way along the under-surface of the shaft of the phallus, while the proximal part of the ostium appears to have been closed by the fusion of the urethral folds. The summit of the glans is marked by a clearly defined pit. The scrotal swellings are hemispherical and are situated one on either side of the midline just cranial to the base of the phallus. The developing testes now contain easily identifiable, eosinophilic interstitial cells, a feature that was absent in the younger specimens.

Cellular buds extending from the lateral walls of the phallic part of the urogenital sinus have appeared. These are the bulbo-urethral gland rudiments, and they originate from the lining of the phallic portion of the urogenital sinus close to its junction with the pelvic part.

The urogenital ostium is still in relation to the most anterior portion of the pars phallica, the epithelial lining of which is very thickened. The urethral folds have fused to close the most caudal part of the pars phallica, thus forming the proximal part of the penile urethra. Owing to the obliquity of the sections, it is possible to confirm that the deeper part of the urethral groove along the shaft of the phallus is formed by the disintegration of the thickened ventral part of the urethral plate. The urethral plate extends into the glans, but does not quite reach the tip, as a short cellular cord of cells derived from the surface epithelium lining the pit on the summit meets the anterior extremity of the urethral plate.

At 35 mm. the external genitalia and their histological picture are very similar to those seen at the 32 mm. stage, and these two stages may be described together. The phallus is bent to a right angle and is 3 mm. long (Pl. 2, fig. 22). The most caudal part of the urethral groove has been closed by fusion of the urethral folds, so that the urogenital ostium has been displaced distally along the under-surface of the developing penis and opens into the proximal end of the urethral groove.

The proximal limit of this groove is situated at about a third of the way along the shaft of the phallus and is wide and deep. This wide and deep portion of the groove is continued on to the under-surface of the proximal part of the glans, and from there on the groove becomes narrower and shallower as it is traced towards the terminal pit near the summit of the glans.

In previous specimens, the part of the abdominal wall between the base of the developing phallus or penis was more or less translucent and consisted of surface epithelium covering loose mesenchyme. The corresponding region in these foetuses is more or less equally divided by a raised crescentic margin disposed transversely to the cranio-caudal axis of the foetus. The part of the developing penis containing the formed (closed) portion of the penile urethra is seen to be attached to the ventral abdominal wall caudal to the crescentic margin. Thus the shaft of the penis has begun to be incorporated in the abdominal wall, and as its free portion is being drawn towards the umbilicus the genital swellings have come to lie caudal to the base of the free portion of the phallus.

In the testes of both specimens the interstitial cells are large, very well defined and eosinophilic.

The phallic portion of the urogenital sinus, the lining epithelium of which is many cells thick, and the proximal part of the urethral groove have been closed and converted into a tube by fusion of the urethral folds in the midline. The resulting raphe is indicated on the surface by a ridge. The folds fuse in such a way that epithelium derived from the sinus or the urethral plate forms the lining of the resulting urethra, and surface epithelium is excluded except possibly from its floor (Pl. 1, fig. 10). The proximal portion of the urethral groove is lined by epithelium derived from the lower basal part of the urethral plate (Pl. 1, fig. 12). The site of junction with surface epithelium is indicated by a heaping up of cells inside the free margin of the urethral folds. In the glans the urethral plate is met by an ingrowth of surface cells along the under-surface of the glans. The site of junction of the two types of epithelium results in a proliferation, which by breaking down gives rise to the distal part of the urethral groove (Pl. 1, fig. 12), which is encroaching on the glans of both specimens. The ingrowth of surface cells referred to above is continuous with the cord of cells which grows in from the terminal pit (Pl. 1, fig. 10) to reach the distal part of the urethral plate.

Sections through the abdominal wall show that the crescentic margin, described in conjunction with the appearances of the external genitalia, is the surface indication of the inner limit of the caudal part of a ring of differentiating subcutaneous muscle. This ring is disposed around the attachment of the umbilical cord to the abdominal wall, but the ring is still separated from the cord by a wide cuff of loose mesenchyme. The subcutaneous muscle ring is the developing umbilical sphincter, and it lies superficial to the differentiating abdominal subcutaneous muscle sheet. The formed parts of the developing penis are closely attached to the developing sphincter, the junctional region being marked by numerous capillaries (Pl. 2, fig. 17).

At 45 mm. the penis is clearly subdivided into two unequal parts (Pl. 2, fig. 23), the total length being about 7 mm. The larger segment comprises that part of the penis which is enclosed in the body wall, the smaller segment is free and recurved

and consists mainly of an elongated glans; a fold of skin extends round the back and sides of its base. The extent of the larger 'pars captiva' is indicated on the surface by a median raphe. The base of the 'pars liber' is situated half-way between the scrotal swellings and the attachment of the umbilical cord. The free portion of the penis is so recurved that it is impossible to see where the urinary meatus is situated. The raised margin on the caudal part of the abdominal wall of the previous two specimens consisted of an arc of a circle around the umbilical cord. The corresponding margin in the 45 mm. foetus, although still separated from the umbilical cord by a depressed area 1 mm. wide, encircles the attachment of the cord to the abdominal wall completely. The fold of skin surrounding the base of the free portion of the penis joins the latter to the most caudal point on the circumference of the raised margin. The fold corresponds to the penile basal ring described in various ungulate foetuses by Broman in 1947.

The epithelium lining the part of the urethra derived from the urogenital sinus is still very thick, and the bulbo-urethral gland rudiments now consist of a solid duct leading to ramifying cellular buds. The duct joins the proximal part of the penile urethra near its junction with the membranous urethra (Pl. 1, fig. 9). The penile urethra is fully formed as far as the glans except for the persistence of the urethral plate in the roof of the most distal part. The line of fusion of the urethral folds is indicated by a fibrous condensation extending from the floor of the urethra to the thickened raphe on the surface of the foetus. The urinary meatus is situated on the under-surface of the glans. The roof of the proximal part of the urethral groove and the most distal part of the formed urethra are lined by epithelium derived from the urethral plate (Pl. 2, figs. 13, 14). This epithelium is less thick than that lining the parts of the urethra derived from the phallic part of the urogenital sinus. Along the inner surface of the urethral folds, the junction between surface epithelium and epithelium derived from the urethral plate is indicated by a heaping up of epithelial cells. Closure of the folds may result in the inclusion of some surface cells in the lining of the floor of this portion of the urethra, where the epithelium is many cells thicker than in the roof. The urethral plate extends into the proximal portion of the glans and the ventral (superficial), reactive, thickened part of it breaks down and leaves epithelial bridges across the resulting groove. The dimensions of the surface ingrowth to meet the distal part of the urethral plate have increased greatly, and the reactive proliferation at this junctional region is breaking down to form the most distal part of the urethral groove (Pl. 2, fig. 15).

Although it was not evident to the naked eye, examination of sections through the penis shows that a preputial fold has appeared round the base of the glans, and it lies within the inner surface of the penile basal ring (Pl. 2, fig. 18). Muscle fibres are differentiating to form a fibromuscular sling connecting the umbilical sphincter to the deep aspect and sides of the distal end of the enclosed part of the penis. This sling also extends round the sides of the penis to reach the penile basal ring and the preputial fold.

At 65 mm. the penis has been drawn up to within 1 mm. of the umbilical cord, apparently by the contraction of the umbilical sphincter which invests the cord fairly closely. The penis is 10 mm. long and only the terminal 1 mm. is recurved

and free. The median raphe forms a distinct ridge on the surface of the embryo and overlies the enclosed portion of the penis.

The penile urethra has been formed as far as the proximal part of the glans on whose under-surface the urinary meatus is situated near the tip of the organ. There is no sign of a urethral plate in this specimen, but the epithelium lining the proximal and distal parts of the penile urethra is thicker than in previous specimens. The surface ingrowth from the tip of the glans is a well-formed lamella, the ventral and superficial part of which gives rise to the terminal part of the urethra.

There is a well-developed glandar lamella which grows in alongside the part of the glans which is already enclosed in the abdominal wall. The deeper parts of the lamella are more cellular and less well differentiated than the more superficial parts. The preputial fold and the penile basal ring have merged to form one fold surrounding the superficial part of the glandar lamella. A preputial diverticulum, which possesses a lumen, extends dorsal to the glans from the superficial part of the glandar lamella.

At 85 mm. the penis has been drawn up to the umbilicus (Pl. 2, fig. 24), and the scrotal swellings have migrated caudally to lie just cranial to the anus; they are unfused. The glans of the penis is almost completely covered by the prepuce and a surface indication of the underlying umbilical sphincter is only just visible.

The proximal part of the penile urethra, i.e. the part formed from the phallic portion of the urogenital sinus, is dilated and has a considerably wider lumen than that of the remainder of the penile urethra; its lining epithelium is also stratified to a greater extent. The glandar urethra has been formed to the very tip of the glans. The terminal part of the urethra is derived from the surface ingrowth which grew in to meet the distal part of the urethral plate, which has however disappeared. The ingrowth is lamellar in form but, owing to the twisting of the apical part of the glans, the lamella has also got twisted and the histological picture is somewhat confused. However, it can be seen that it is the superficial basal part of the lamella from which the terminal urethra is derived. The stratified epithelium lining the distal part of the glandar urethra is many cells thick and is similar to and continuous with the epithelium covering the surface of the glans (Pl. 2, fig. 16). The greater distal part of the glandar urethra appears to be lined by this type of epithelium.

The preputial diverticulum opens into the most cranial portion of the preputial furrow. The terminal part of the glans lies twisted within the preputial space which appears to form without the formation of 'epithelial pearls', which are so prominent in the glandar lamella of man. Nor have epithelial pearls been noted in the formation of the glandar urethra, cf. man (Glenister, 1954). The deepest part of the glandar lamella is made up of cells which appear to be less differentiated than those constituting the more superficial parts of the lamella and thus gives the impression of still growing in.

The muscular sling which surrounds the distal part of the enclosed portion of the penis in this specimen, as in the previous two, connects this part of the penis to a ring of muscle around the umbilical cord (Pl. 2, fig. 19).

A dissection of the peri-umbilical region of a 125 mm. foetus and serial sections through the corresponding region of another foetus of the same size, show that

the terminal portion of the penis, which is now completely enclosed within the abdominal wall, is attached to the umbilical sphincter (Pl. 2, fig. 21). The latter lies subcutaneously and forms a sling passing caudal to the umbilical cord and extending cranially as two muscular columns (Pl. 2, fig. 20), flanking either side of the umbilicus to be attached to the cranial portion of the linea alba. Muscle fibres can be traced from the caudal part of the umbilical sphincter to the prepuce, and to the sides of the penis; some extend round the latter to reach the penile raphe.

It should be noted that the cranial portion of the umbilical sphincter lies superficial to the subcutaneous abdominal muscles, and that these two muscles lose their intimate relation to the sphincter caudal to the umbilicus as they diverge towards the region of the stifles.

Although the sphincter and the subcutaneous muscles are attached to the cranial part of the linea alba, they are otherwise superficial to and quite distinct from the rectus sheaths (Pl. 2, fig. 21).

Dissection of the abdominal wall of a near-term foetus shows that the same topographical relations exist up to the time of birth.

DISCUSSION AND CONCLUSIONS

It is evident from this study that although the penis of the pig becomes incorporated in the abdominal wall in the course of development, the penile urethra is formed in a way that is essentially similar to that taking place in man.

The urethral plate develops as a lamellar outgrowth from the fused anterior walls of the cloaca and urogenital sinus. The plate is not, as suggested by Retterer (1914), the product of an outgrowth of urethral folds on either side of a median strip of surface epithelium, continuous with the cloacal membrane. This fact is emphasized by the observation that the urethral plate is recognizable as a well-defined lamella in the 11, 16 and 18 mm. pig embryos; the urethral folds, however, are not clearly seen on the surface till the 20 mm. stage. Although essentially similar to the urethral plates observed in human foetuses, the plates described in these pig foetuses do not extend so deeply into the mesenchyme of the developing penis and thus appear shorter in transverse section.

As may be appreciated from a review of the literature, few, if any, of the authors have studied the details of the formation of the urethral groove in man or in animals, and they seem to have dismissed it as resulting quite simply from the outgrowth of urethral folds on either side of the midline (Debière, 1883; Retterer, 1890-1915; Fleischmann, 1902-7; Tournoux, 1889; Schwartztrauber, 1904; Böhm, 1905; Lichtenberg, 1906; Paschkis, 1906; Johnson, 1920; Spaulding, 1921).

Herzog (1904) implied that it was formed by a splitting of the urethral plate, and only gave rise to the urethra of the glans. Felix (1912) held the same opinion. Van den Broek (1909, 1910) also considered that the superficial part of the urethral plate splits but contributes to the lining of all parts of the urethral groove. Williams (1952) describes a urethral groove on the glans analogous to that of the shaft and derived in part by the 'opening up' of the urethral plate and in part from epidermis.

In fact, the urethral groove is formed in the pig by the same complicated processes

as already described in man (Glenister, 1954). The processes start at the base of the phallus and then extend distally towards the tip. Urethral folds grow out on either side of the urethral plate and on either side of the phallic part of the urogenital sinus, thus forming the *primitive* or *primary urethral groove*. This groove is continuous caudally with the primitive urogenital ostium. The lower, superficial margin of the urethral plate becomes thickened as the surface epithelium in relation to it appears to disintegrate. In turn, this thickening of the urethral plate is followed by a disintegration of the thickened portion to form a *secondary urethral groove*. The combined product of primary and secondary urethral grooves may be termed the *definitive urethral groove*.

However, in the pig fetuses examined, the surface epithelium in contact with the basal margin of the urethral plate, in the roof of the urethral groove, has never been observed to be proliferating before retrogressing and breaking down. This provides an explanation for the fact that no terminal epithelial tags, so prominent in human specimens, have been observed in pig fetuses.

As in man, closure of the urethral folds related to the phallic part of the urogenital sinus gives rise to the proximal dilated portion of the penile urethra, while the remainder of the urethra of the shaft of the penis and of the proximal part of the glandar urethra is formed by the fusion of the part of the urethral folds related to the definitive urethral groove. The fusion of the urethral folds takes place in such a way that surface epithelium is excluded from the lumen of the greater part of the penile urethra. Near the tip of the penis surface epithelium is incorporated into the lining of the floor of the urethra, while the terminal portion of the urethra is derived entirely from surface epithelium which grows in to meet the distal extremity of the urethral plate.

Fusion of the urethral folds gives rise to the perineal raphe. The formation of this structure was described correctly by Retterer (1890–1914), and no evidence has been found to support the views of Tourneux (1888, 1889) and Fleischmann (1902–7) and their respective schools. It should also be noted that the fusion of the urethral folds starts caudally and proceeds towards the tip of the phallus, there being again no evidence to support the views of Rathke (1832).

Attention has already been drawn to the fact that no terminal epithelial tag has been observed in any of the pig fetuses examined. Instead, a terminal pit, which is continuous with the distal part of the urethral groove, appears at about the 20 mm. stage and an epithelial ingrowth, derived from the lining of this pit and the distal part of the urethral groove, grows into the substance of the glans to meet the distal extremity of the urethral plate. In the previous publication dealing with the development of the penile urethra in man it was stated that when the ectodermal epithelium breaks down after proliferation, it does so by passing through a phase of 'epithelial pearl' formation, whereas the urethral plate proliferation breaks down, leaving 'epithelial bridges' across the resulting groove. The latter phenomenon has also been observed in the pig, but the former was absent both in the ingrowth forming the terminal urethra and in the glandar lamella, and could not be used as a criterion of surface origin.

As regards the formation of the prepuce it would appear to start soon after the 40 mm. stage (a preputial fold is present in the 45 mm. specimen) at which stage

the glans is quite free from the abdominal wall. By the 65 mm. stage, the glans has been partly incorporated into the abdominal wall and the processes of prepuce formation are thereby obscured. However, the glandar lamella is well developed by this stage, and at the 85 mm. stage, when the deeper portions of the lamella still appear to consist of less well-differentiated ingrowing cells. It seems therefore that, in the pig, the prepuce results from a combination of preputial fold formation and ingrowth of a glandar lamella; in other words, by processes essentially similar to those taking place in human foetuses (it is hoped to publish a note on the development of the prepuce in man at a later date).

Thus the views expressed here concur with those of Retterer (1890-1915), who believed that prepuce formation results from a combination of preputial fold and glandar lamella formation. It may be added that the observations described in this paper could not be interpreted satisfactorily were the prepuce to result from preputial fold formation only, as claimed by Berry Hart (1908) and Hunter (1935), or to result entirely from the ingrowth and subsequent splitting of the glandar lamella as described by Böhm (1905), Wood Jones (1910) and Johnson (1920).

The incorporation of the penis in the abdominal wall and the drawing up of the penile tip to the umbilical region would seem to result from an early association of the formed parts of the penis with the developing umbilical sphincter. The onset of the process of incorporation coincides with the appearance in the abdominal wall of a subcutaneous umbilical sphincter to which the formed part of the penis becomes attached by a fibromuscular sling. This sling extends from the most caudal part of the sphincter to the sides of the penis and to the line of fusion of the urethral folds. As the muscle ring constituting the sphincter comes to encircle the attachment of the umbilical cord more closely, the distal end of the formed part of the penis is drawn forward progressively so that the tip ultimately lies just caudal to the umbilicus. As well as extending round the sides of the penis to reach the line of fusion of the urethral folds, the fibromuscular sling connecting the penis to the umbilical sphincter can also be traced to the preputial fold and the penile basal ring when these structures appear. Broman (1947) claimed that this penile basal ring connected the base of the free portion of the developing penis to a margin raised by the inner edge of the developing rectus muscles and that the basal ring was responsible for drawing up the penile tip to the umbilicus. It must be pointed out that Broman only examined his material macroscopically. When ungulate foetuses are sectioned and examined microscopically, it becomes obvious that the raised margin is the surface indication of the underlying umbilical sphincter, and that the penile basal ring is a cutaneous fold to which some of the fibres of the connecting sling are attached. This fold later merges with the preputial fold to form the superficial distal part of the prepuce, which is drawn over the tip of the penis when the latter becomes completely enclosed in the abdominal wall.

It should be noted that all the while part of the urethral groove remains open, the penile basal ring and preputial fold remain caudal to the open part of the groove and the urogenital ostium which opens into it. In other words, the successive parts of the penis are incorporated into the abdominal wall only after the part of the urethra related to them has been formed.

Thus Zietschmann's account (1924), describing the cranial migration of the

phallus as being caused by a lengthening of the axial parts of the organ under the perineal skin, is not accurate.

The bulbo-urethral glands of the pig develop, as in man, as cellular outgrowths from the phallic part of the urogenital sinus near its junction with the pelvic portion.

This study may thus be said to indicate that an ungulate such as the pig develops its penile urethra by processes essentially similar to those taking place in man, but complicated by the attachment of the penis to an umbilical sphincter, a structure not found in human fetuses (Parry, 1954).^{*} The uniformity of the pattern of urethral development described here conforms to the view of Retterer (1914), who stated that all mammals develop their external genitalia according to the same basic plan.

In conclusion, attention must be drawn to the marked proliferation and stratification observed in the lining epithelium of the parts of the urethra developed from the urogenital sinus and in the glandular urethra, whereas the intermediate portion shows no such phenomenon.

The proliferation is very reminiscent of that described by Zuckerman (1940), Moore (1941) and Burns (1942), in connexion with the modifications induced by oestrogens in epithelium derived from the urogenital sinus. The proliferation observed in the present series, as also in the untreated specimens described by Burns, could conceivably be due to the oestrogens of the mother. It is, however, difficult to understand why, on the one hand, a lining epithelium derived from the surface of the embryo and one derived from the urogenital sinus should react, whereas the intermediate portion derived from the urethral plate and so also ultimately from the sinus, does not. The possibility that the response to oestrogens is not as specific as suggested by Zuckerman (1940) and Burns (1942) must be envisaged.

It must be conceded to these authors when considering from which germ-layer the lining of the urogenital sinus and the urethral plate are derived, that the possibility exists that ectodermal cells may be incorporated in their formation. The cloacal membrane gives rise to the floor of the urogenital sinus, and may possess a degree of plasticity which enables ectodermal cells to migrate to the deeper part of the membrane and so become incorporated in the primarily endodermal lining of the sinus. This incorporation must, however, take place very early in development, before the breaking down of the cloacal membrane and not after this, as suggested by Burns (1942). The replacement of one type of epithelium by another, which he observed in his experimental material and suggested took place in normal fetuses after the breakdown of the membrane, has not been observed in either man or the pig.

^{*} Hauptmann (1911) described an umbilical sphincter in the horse, and Parry (1954) described this structure in the cow, sheep, rabbit and guinea-pig. She noted the absence of the sphincter in man, macaque, rat, dog, cat and pangolin. She stated that her preliminary observations indicated the presence of an umbilical sphincter in the pig. As the result of this investigation it is confirmed that the pig possesses this structure, and dissection of near-term fetuses shows that the deer and the springbok may be added to the list of mammals possessing an umbilical sphincter. In their case, as in the case of the cow, sheep and pig, the terminal part of the penis and the prepuce are attached to the sphincter.

The suggestion that the distal part of the urogenital sinus is histogenetically related to ectoderm and that there may be a tendency for these distal ectodermal components of the sinus to impress their character on the proximal endodermal tissue (Zuckerman, 1940) is based on the accounts of Keibel (1910), Pohlman (1911), Lewis (1912), Keith (1923) and Frazer (1931), according to which the cloacal membrane incorporates part of the primitive streak. As the streak is considered to be the homologue of the blastopore of lower forms, the cloacal zone is looked upon as a region which, in an earlier phase of its development, was the centre of formative movements of the body and where cells streamed in from the surface of the embryo to deeper structures.

Attractive as this hypothesis may be when seeking an explanation for the results of experimental embryology, it must be remembered that in the first place the cells which stream in from the surface in the region of the primitive streak are cells giving rise to mesodermal components. Secondly, Florian (1930, 1933, 1934) and Wyburn (1937) have demonstrated that, at any rate in man, the cloacal membrane is not a secondary formation, but that the fusion of ectoderm and endoderm at the caudal end of the embryonic shield occurs as early as, if not before, the appearance of the primordium of the primitive streak. According to these authors, the streak represents the fused lips of the blastopore, the cloacal membrane being the homologue of the ventral lip of the blastopore and the adjacent area. Neither author mentions any streaming in of surface cells to deeper structures within the confines of the cloacal membrane. Wyburn considers, further, that the cloacal membrane is not so much a development as an actual persistence of contact between ectoderm and endoderm, the contact not being disturbed by mesoderm passing round the membrane towards the body stalk from the primitive streak. According to this author the streak ends at or short of the cloacal membrane.

SUMMARY

1. A series of pig foetuses ranging in size from 11 to 125 mm. crown-rump length has been examined to ascertain whether incorporation of the penis in the abdominal wall affects the processes of development of the penile urethra.
2. The penile urethra of the pig has been found to develop by processes essentially similar to those already described by the author in man.
3. The drawing forward of the penile tip to the umbilical region is effected by a fibromuscular attachment of the distal part of the penis to the umbilical sphincter.
4. The relative importance of the contributions of ectoderm and endoderm to the urethral lining is discussed.

I wish to acknowledge the encouragement and constructive criticism received from Prof. W. J. Hamilton. I am indebted to Mr R. H. Watts for his technical assistance and to Mr R. J. McCulloch for the photography.

REFERENCES

- BISCHOFF, T. L. W. (1842). *Entwicklungsgeschichte der Säugethiere und des Menschen*. Leipzig: Sömmerring.
- BÖHM, J. (1905). Die äusseren Genitalien des Schafes. *Morph. Jb.* **34**, 248–320.
- BROMAN, I. (1945). Entsteht der Penis pendulus wirklich durch 'Lösung' von der Bauchwand? *Acta anat.* **1**, 66–71.
- BROMAN, I. (1947). Über die Entstehung und sekundäre Verschiebung der äusseren Geschlechts-teile bei Wiederkäuern. *Acta anat.* **3**, 15–54.
- BURNS, R. K. (1942). The origin and differentiation of the epithelium of the urogenital sinus in the opossum, with a study of the modifications induced by oestrogens. *Contr. Embryol. Carneg. Instn.* **30**, 68–83.
- CADIAT, O. (1884). Du développement du canal de l'urètre et des organes génitaux de l'embryon. *J. Anat., Paris*, **20**, 242–264.
- DEBIÈRE, CH. (1883). Développement de la vessie, de la prostate et du canal de l'urètre. Thèse d'agrégation, Faculté de Médecine de Paris: Doin.
- DURBECK, W. (1907). Die äusseren Genitalien des Schweines. *Morph. Jb.* **36**, 517–543.
- DURBECK, W. (1907). Die äusseren Genitalien der Hauskatze. *Morph. Jb.* **36**, 544–565.
- DURBECK, W. (1907). Tabellarische Übersicht der Genitalentwicklung bei Säugetieren. *Morph. Jb.* **36**, 566–569.
- FELIX, W. (1912). *The Development of the Urogenital Organs*. In *Manual of Human Embryology*, 2, ed. by F. Keibel and F. P. Mall. Philadelphia and London: Lippincott.
- FLEISCHMANN, A. (1902–7). Morphologische Studien über Kloake und Phallus der Amnioten. IV. Die Säugetiere. *Morph. Jb.* **30**, 653–666.
- FLEISCHMANN, A. V. Die Stilistik des Urodäums und Phallus bei den Amnioten. *Morph. Jb.* **30**, 666–675.
- FLEISCHMANN, A. VII. Historisch-kritische Betrachtungen. *Morph. Jb.* **32**, 58–96.
- FLEISCHMANN, A. VIII. Die Stilistik des Urodäums. *Morph. Jb.* **32**, 97–103.
- FLEISCHMANN, A. XVI. Die Stilcharaktere am Urodäum und Phallus. *Morph. Jb.* **36**, 570–601.
- FLORIAN, J. (1930). The formation of the connecting stalk and the extension of the amniotic cavity towards the tissue of the connecting stalk in young human embryos. *J. Anat., Lond.*, **64**, 454–476.
- FLORIAN, J. (1933). The early development of man, with special reference to the development of the mesoderm and cloacal membrane. *J. Anat., Lond.*, **67**, 263–276.
- FLORIAN, J. (1933). The scheme of development of the axial formation in the human embryo up to the stage with 10 pairs of somites. *Čas. Lék. čes.* **72**, 1375–1378.
- FLORIAN, J. (1934). Ein Schema der Entwicklung der Axialgebilde des menschlichen Embryos bis in das Stadium von 10 Urvirbelpaaren. *Biol. gen.* **10**, Lief. 2.
- FRAZER, J. E. (1931). *Manual of Embryology*, 1st ed. London: Baillière, Tindall and Cox.
- GILLMAN, J. (1948). The development of the gonads in Man, with a consideration of the role of fetal endocrines and the histogenesis of ovarian tumors. *Contr. Embryol. Carneg. Instn.* **32**, 81–131.
- GLENISTER, T. W. (1954). The origin and fate of the urethral plate in Man. *J. Anat., Lond.*, **88**, 413–425.
- HART, D. BERRY (1908). On the role of the developing epidermis in forming sheaths and lumina to organs. Illustrated specially in the development of the prepuce and urethra. *J. Anat., Lond.*, **42**, 50–56.
- HAUPTMANN, E. (1911). Über den Bau des Nabelstranges beim Pferde mit besonderer Berücksichtigung der natürlichen Rissstelle. *Arch. Anat. Physiol., Lpz. (Abt. Anat.)*, pp. 103–132.
- HERZOG, F. (1904). Beiträge zur Entwicklungsgeschichte und Histologie der männlichen Harnröhre. *Arch. mikr. Anat.* **63**, 710–747.
- HUNTER, R. H. (1935). Notes on the development of the prepuce. *J. Anat., Lond.*, **70**, 68–75.
- JOHNSON, F. P. (1920). The later development of the urethra in the male. *J. Urol.* **4**, 447–493.
- JONES, F. WOOD (1910). The development and malformations of the glans and prepuce. *Brit. med. J.* **1**, 137–138.
- KEIBEL, F. (1910). In *Manual of Human Embryology*, 1, ed. by F. Keibel and F. P. Mall. Philadelphia and London: Lippincott.

- KEITH, A. (1923). *Human Embryology and Morphology*. London: Edward Arnold.
- KOELLIKER, A. (1861). *Entwicklungsgeschichte des Menschen und der höheren Thiere*, II. Leipzig: Engelmann.
- LEWIS, F. T. (1912). In *Manual of Human Embryology*, 2, ed. by F. Keibel and F. P. Mall. Philadelphia and London: Lippincott.
- LICHTENBERG, A. (1906). Beiträge zur Histologie, mikroskopischer Anatomie und Entwicklungsgeschichte des Urogenitalkanals des Mannes und seiner Drüsen. *Arb. anat. Inst., Wiesbaden*, 30, 63-198.
- MECKEL, J. F. (1815). *Handbuch der menschlichen Anatomie*. Halle: Renger.
- MOORE, C. R. (1941). On the role of sex hormones in sex differentiation in the opossum (*Didelphys virginiana*). *Physiol. Zool.* 14, 1-47.
- MÜLLER, J. (1830). *Bildungsgeschichte der Genitalien*. Düsseldorf: Arnz.
- PARRY, M. J. (1954). A comparative study of the umbilical sphincter. *Proc. zool. Soc. Lond.* 124, 595-604.
- PASCHKIS, R. (1906). Zur Anatomie und Entwicklungsgeschichte der männlichen Harnröhre. *Mber. Urol.* 9, 641-662.
- POHLMAN, A. G. (1911). The development of the cloaca in human embryos. *Amer. J. Anat.* 12, 1-26.
- RATHKE, H. (1832). *Abhandlungen zur Bildungs- und Entwicklungsgeschichte des Menschen und der Thiere*. Tl. 1, Leipzig.
- RETTERER, E. (1890). Sur le cloisonnement du cloaque et sur la formation du périnée. *C.R. Soc. Biol., Paris*, 42, 3-7.
- RETTERER, E. (1890). Note sur le développement des organes génitaux externes et de l'anus. *C.R. Soc. Biol., Paris*, 42, 289-292.
- RETTERER, E. (1890). Du développement du prépuce, de la couronne du gland et du col du pénis chez l'embryon humain. *C.R. Soc. Biol., Paris*, 42, 528-531.
- RETTERER, E. (1890). Du développement du fourreau et de la partie libre de la verge des mammifères quadrupèdes. *C.R. Soc. Biol., Paris*, 42, 551-554.
- RETTERER, E. (1890). Note sur le développement de la portion abdominale de la verge des mammifères. *C.R. Soc. Biol., Paris*, 42, 606-608.
- RETTERER, E. (1890). Sur l'origine et l'évolution de la région anogénitale des mammifères. *J. Anat., Paris*, 26, 126-151, 153-216.
- RETTERER, E. (1891). Développement de la double gaine préputiale du cheval. *C.R. Soc. Biol., Paris*, 43, 116-119.
- RETTERER, E. (1892). Sur le développement du pénis et du clitoris chez les fœtus humains. *J. Anat., Paris*, 28, 225-281.
- RETTERER, E. (1905). Du rôle de l'épithélium dans le développement des organes génito-urinaires externes. *C.R. Soc. Biol., Paris*, 58, 1040-1043.
- RETTERER, E. (1905). Du développement et de la structure des raphés des organes génito-urinaires. *C.R. Soc. Biol., Paris*, 59, 22-25.
- RETTERER, E. (1914). Développement et histogenèse comparée des organes génitaux externes. *J. Urol. méd. chir.* 6, 157-173, 327-344.
- RETTERER, E. (1914). Index of Retterer's publications. *J. Urol. méd. chir.* 6, 343.
- RETTERER, E. & NEUVILLE, H. (1915). Développement comparé du gland et du prépuce des singes et de l'homme. *C.R. Soc. Biol., Paris*, 78, 387-390.
- SCHWARTZTRAUBER, J. (1904). Kloake und Phallus des Schafes und Schweines. *Morph. Jb.* 32, 23-57.
- SPAULDING, M. H. (1921). The development of the external genitalia in the human embryo. *Contr. Embryol. Carneg. Instn.* 13, 67-88.
- TIEDEMANN, F. (1813). *Anatomie der kopflosen Missgeburten*. Landshut: Thomann.
- TOURNEUX, F. (1888). Les premiers développements du cloaque, du tubercule génital et de l'anus chez l'embryon du mouton. *J. Anat., Paris*, 24, 503-517.
- TOURNEUX, F. (1889). Sur le développement et l'évolution du tubercule génital chez le fœtus humain dans les deux sexes, avec quelques remarques concernant le développement des glandes prostatiques. *J. Anat., Paris*, 25, 229-263.
- VALENTIN, G. G. (1835). *Handbuch der Entwicklungsgeschichte des Menschen*. Berlin: Rücker and Püchler.

- VAN DEN BROEK, A. J. P. (1909). About the development of the urogenital canal (urethra) in man. *Proc. Acad. Sci. Amst.* **11**, 494-499.
- VAN DEN BROEK, A. J. P. (1910). Über den Schlierungsvorgang und den Bau des Urogenitalkanales beim menschlichen Embryo. *Anat. Anz.* **37**, 106-120.
- WILLIAMS, D. INNES (1952). The development and abnormalities of the penile urethra. *Acta anat.* **15**, 176-187.
- WYBURN, G. M. (1937). The development of the infra-umbilical portion of the abdominal wall, with remarks on the aetiology of ectopia vesicae. *J. Anat., Lond.*, **71**, 201-231.
- ZIETSMANN, O. (1924). *Lehrbuch der Entwicklungsgeschichte der Haustiere*, 3rd ed. Berlin: Schoetz.
- ZUCKERMAN, S. (1940). The histogenesis of the tissues sensitive to oestrogens. *Biol. Rev.* **15**, 231-271.

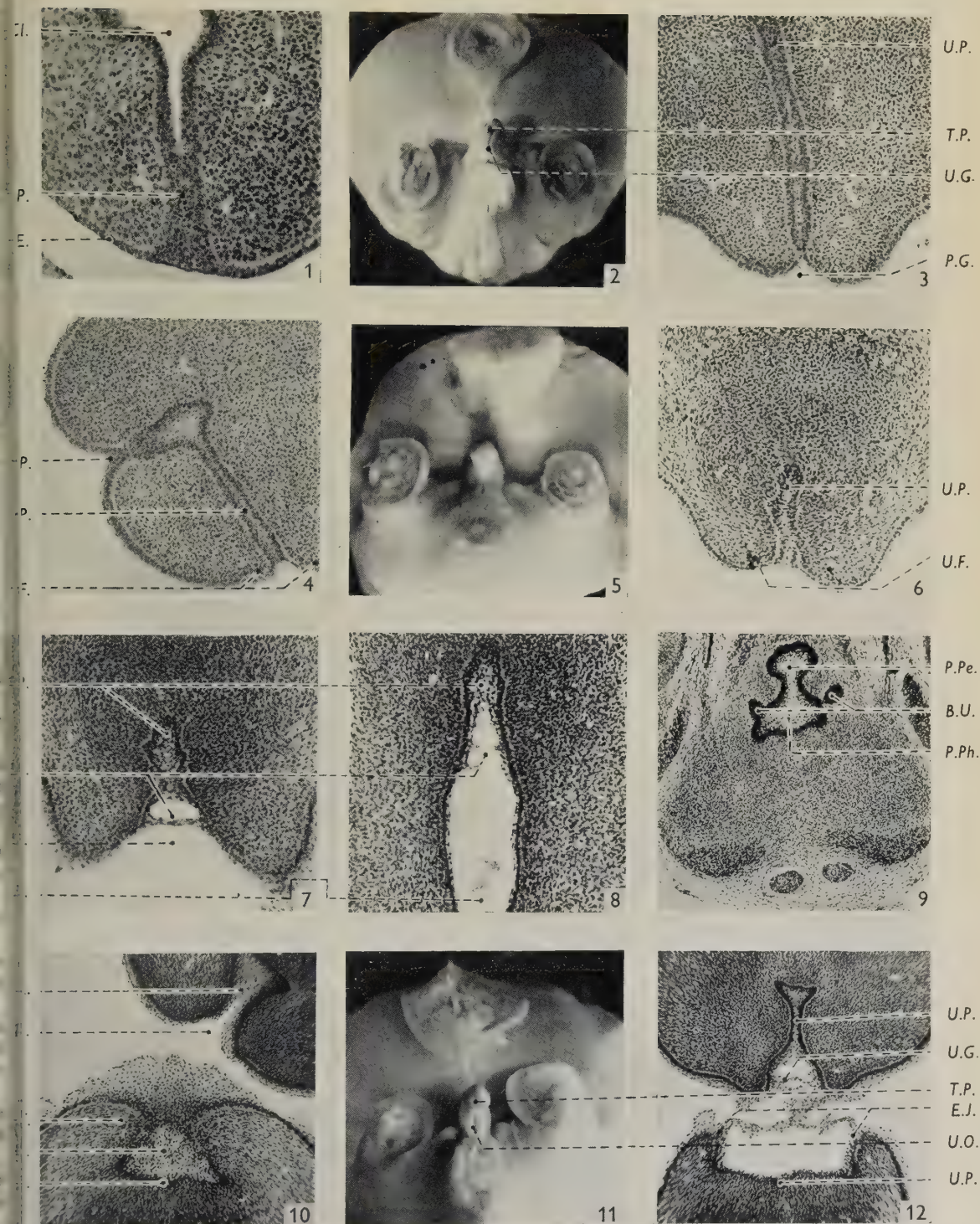
EXPLANATION OF PLATES

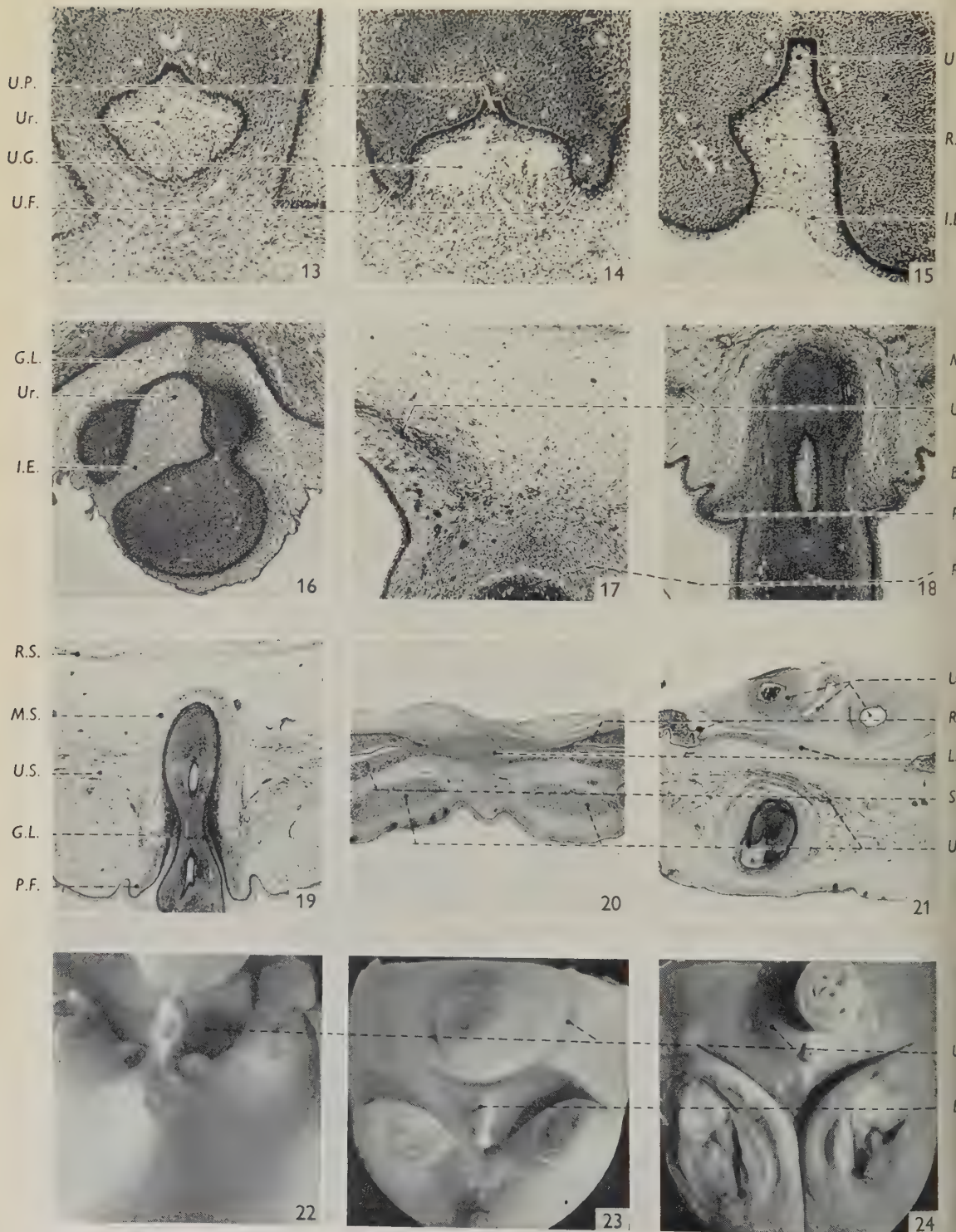
List of abbreviations

<i>B.R.</i>	penile basal ring	<i>P.Ph.</i>	phallic portion of the urogenital sinus
<i>B.U.</i>	bulbo-urethral gland rudiment	<i>R.P.</i>	reactive proliferation
<i>Cl.</i>	cloaca	<i>R.S.</i>	rectus sheath
<i>E.J.</i>	junction of surface epithelium with epithelium derived from the urethral plate	<i>S.E.</i>	surface epithelium
<i>F.P.</i>	free portion of the penis	<i>S.G.</i>	secondary groove
<i>G.L.</i>	glandar lamella	<i>S.M.</i>	subcutaneous muscle
<i>I.E.</i>	ingrowth of surface epithelium	<i>T.P.</i>	terminal pit
<i>L.A.</i>	linea alba	<i>U.F.</i>	urethral fold
<i>M.S.</i>	muscular sling	<i>U.G.</i>	urethral groove
<i>P.F.</i>	preputial fold	<i>U.O.</i>	urogenital ostium
<i>P.G.</i>	primary groove	<i>U.P.</i>	urethral plate
<i>P.Pe.</i>	pelvic portion of the urogenital sinus	<i>Ur.</i>	urethra
		<i>U.S.</i>	umbilical sphincter
		<i>U.V.</i>	umbilical vessels

PLATE 1

- Fig. 1. Transverse section through the cloacal region of the 11 mm. pig embryo. The urethral plate is seen to be formed from the converging walls of the cloaca. $\times 120$.
- Fig. 2. The external genitalia of the 22 mm. pig foetus, showing the urethral groove on the under-surface of the phallus, and the terminal pit. $\times 4.5$.
- Fig. 3. Transverse section through the phallus of the 22 mm. foetus, showing that the urethral plate is distinct from the surface epithelium. The latter is thinned out and retrogressing in relation to the basal portion of the urethral plate. The primary urethral groove is flanked by urethral folds. $\times 87$.
- Fig. 4. Oblique section through the tip of the phallus of the 22 mm. foetus, showing the ingrowth of surface epithelium from the terminal pit to meet the distal extremity of the urethral plate. $\times 87$.
- Fig. 5. The external genitalia of the 25 mm. foetus, showing that the proximal end of the urethral groove is deeper than the distal portion. $\times 4.5$.
- Fig. 6. Transverse section through the distal part of the shaft of the phallus of the 27 mm. foetus. The urethral plate is lamellar in shape, and the surface epithelium lining the primary urethral groove in relation to the lower margin of the plate is thinned out. $\times 87$.
- Fig. 7. Transverse section through the proximal part of the phallus of the 27 mm. foetus, showing that the basal part of the urethral plate is thickened and disintegrating. The resulting secondary groove has epithelial bridges extending across it, and it deepens the primary groove which is lined by surface epithelium. $\times 75$.
- Fig. 8. Coronal section through the caudal parts of the 27 mm. foetus, showing (from above downwards) the urethral plate, the most caudal part of the secondary urethral groove and the phallic part of the urogenital sinus. $\times 75$.
- Fig. 9. Coronal section through the junction of the pelvic and phallic portions of the urogenital sinus of the 45 mm. foetus. The bulbo-urethral gland rudiments are seen extending from the phallic part. $\times 48$.





GLENISTER—THE DEVELOPMENT OF THE PENILE URETHRA IN THE PIG

- Fig. 10. Transverse section through the curved phallus of the 35 mm. foetus. The distal segment is seen in the upper part of the figure, and it shows the ingrowth of surface epithelium from the terminal pit. In the proximal segment, the urethral folds are seen approaching one another to convert the urethral groove into a tubular urethra. $\times 60$.
- Fig. 11. The external genitalia of the 32 mm. foetus. The urethral folds have fused to close the most caudal portion of the phallic part of the urogenital sinus. As the result of this, the urogenital ostium is no longer situated at the base, but now opens on the under-surface of the phallus. $\times 4.5$.
- Fig. 12. Transverse section through the curved phallus of the 35 mm. foetus. The distal segment is seen in the upper part of the figure and shows the reactive proliferation at the lower margin of the urethral plate, breaking down to give rise to the urethral groove on the glans. The urethral groove on the shaft of the phallus (lower part of figure) is lined by cells derived from the urethral plate. The junction of this type of epithelium and surface epithelium is indicated by a heaping up of cells. $\times 60$.

PLATE 2

- Fig. 13. Transverse section through the distal part of the formed penile urethra of the 45 mm. foetus. The remains of the urethral plate are seen in the roof of the urethra. $\times 90$.
- Fig. 14. Transverse section through the urethral groove of the 45 mm. foetus. Only the roof of the groove is lined by cells derived from the urethral plate. $\times 90$.
- Fig. 15. Transverse section through the distal part of the penis of the 45 mm. foetus, showing the reactive proliferation at the junction of the urethral plate and surface ingrowth. The proliferation is breaking down to give rise to the most distal part of the urethral groove. $\times 90$.
- Fig. 16. Transverse section through the distal part of the penis of the 85 mm. foetus, showing that the lining of the terminal part of the urethra is similar to and continuous with the superficial lining of the penis and the glandular lamella. $\times 45$.
- Fig. 17. Transverse section through the distal part of the enclosed portion of the developing penis of the 38 mm. foetus. The region of attachment of the penis to the developing umbilical sphincter is marked by numerous arterioles and capillaries. $\times 39$.
- Fig. 18. Transverse section through the junction of the free and enclosed parts of the penis of the 45 mm. foetus. The preputial fold and the penile basal ring outside it are seen flanking the base of the free portion of the penis. A fibromuscular sling joins these cutaneous folds and the sides of the penis to the developing umbilical sphincter. $\times 36$.
- Fig. 19. Transverse section through the distal part of the penis of the 85 mm. foetus, showing the relations of the penis and fibromuscular sling to the umbilical sphincter and of the latter to the rectus sheath and linea alba. $\times 15$.
- Fig. 20. Transverse section through the abdominal wall of the 125 mm. foetus, showing the relations of the umbilical sphincter to the subcutaneous muscle sheet, the rectus sheath and the linea alba. $\times 12$.
- Fig. 21. Transverse section through the abdominal wall of the 125 mm. foetus at a level which is caudal to that of Fig. 20. This figure shows the relations of the terminal portion of the penis to the umbilical sphincter. $\times 12$.
- Fig. 22. The external genitalia of the 38 mm. foetus. The proximal part of the penis has become attached to the abdominal wall. The junction between the free and attached portions of the penis is connected to the margin raised by the underlying developing umbilical sphincter. $\times 4.5$.
- Fig. 23. The external genitalia of the 45 mm. foetus showing the penile basal ring connecting the base of the free portion of the penis to the margin raised by the developing umbilical sphincter. $\times 3.6$.
- Fig. 24. The external genitalia of the 85 mm. foetus showing that the umbilical sphincter encircles the umbilical cord closely and has drawn up the penile tip to within a fraction of a millimetre caudal to the cord. $\times 3.6$.

THE UPTAKE OF RADIOACTIVE SULPHATE BY CELLS, FIBRES AND GROUND-SUBSTANCE OF MATURE AND DEVELOPING CONNECTIVE TISSUE IN THE ADULT MOUSE

By A. GLÜCKSMANN,* ALMA HOWARD AND S. R. PELC†

The uptake of labelled sulphate by connective tissue has been noted by a number of authors (Belanger, 1954; Bostrom & Jorpes, 1954; Campbell & Persson, 1951; Davies & Young, 1954), but not studied in detail in the adult animal. We observed a differential uptake of labelled sulphate in the stroma of various glandular organs (epididymis, testis and seminal vesicle) in the adult mouse which might be supposed to be related to the activity of the organ. Before putting forward such a hypothesis, it was decided to study the uptake of labelled sulphate in mature and developing connective tissue in the adult mouse, in order to find whether this uptake is related to specific stages in the development of fibrous tissue, and whether it is primarily cellular or extra-cellular.

MATERIAL AND METHODS

Two series of experiments were made: (1) Labelled sulphate ($^{35}\text{SO}_4$) was injected into adult mice and the animals killed at intervals after the injection; the uptake, retention and loss of sulphate were studied by means of autoradiographs (*timing experiment*). This permitted the study of the uptake and subsequent movement of sulphate in adult connective tissue and mast cells. The dermis of the dorsal skin region and of the pinna, and the substantia propria of the cornea were used. (2) Standard wounds were made in adult mice (*wound-healing experiment*) and the animals killed at various intervals after making the wounds; the radioactive sulphate was always injected 24 hr. before the animal was killed. Stages in the formation of the collagen fibre bundles were examined after a constant interval following sulphate injection.

Eleven male and nineteen female F_1 hybrid mice (C_{57} Black \times C_3H) and twenty-one male and nine female C_3H mice were used in the experiments. Labelled sulphate in the form of $\text{Na}_2^{35}\text{SO}_4$ was obtained from the Isotope Division, A.E.R.E., Harwell, and diluted with distilled water to a concentration of $500\mu\text{C}$. per ml. 0.5 ml. per animal was injected intraperitoneally. In the wound-healing experiment a standard wound was made in the dorsal skin under ether-anaesthesia by cutting a circular hole of 5 mm. diameter (Dann, Glücksmann & Tansley, 1941, 1942). The wound was covered with penicillin powder.

Tissues were fixed for 1 hr. in acetic acid-alcohol (1 : 3), then transferred to formol saline (1 : 8) for 24 hr., embedded in paraffin wax and sectioned at 5μ .

* Working with a grant from the British Empire Cancer Campaign at the Strangeways Research Laboratory, Cambridge.

† Experimental Radiopathology Research Unit of the Medical Research Council, Hammer-smith Hospital, London.

Autoradiographs were prepared by the stripping film technique (Doniach & Pelc, 1950), which gives a resolving power of $2-3\mu$ when ^{35}S is used as a tracer. The slides were exposed for 15 or 16 days, and developed at $17.5-18^\circ\text{C}$. for 5 min. in Kodak D 19 C developer, rinsed in distilled water and fixed in dilute acid fixer for 10 min.

Observations were made either on unstained preparations with the phase-contrast microscope or on preparations stained, after photographic processing, with carmalum, carmalum and neutral red or toluidine blue.

Control slides were stained by the following methods: Lillie's periodic acid Schiff (PAS) method, a modified azan-stain, van Gieson, Weigert's elastic stain or Wilder's silver stain. All these staining techniques were applied after fixation in acetic-alcohol, followed by formol fixation. The connective tissue gave a pink PAS stain which was unchanged after diastase digestion, and which contrasted with the more intense staining of the basement membrane of the epidermis and the hair follicles. In the connective tissue only the mast cells gave a strongly metachromatic stain with toluidine blue.

TIMING EXPERIMENT

Our observations are concerned mainly with the cells and extracellular and fibrous material of the dermis in the interscapular region, the ear and the substantia propria of the cornea. Uptake is, of course, not restricted to these structures and is found, for example, in some sebaceous glands and hair sheaths and also slightly in the dermal muscle.

In animals fixed 2 hr. after the injection of sulphate, autoradiographs are observed over the cells, the fibres and the inter-fibrillar material of the connective tissue in all three sites (Pl. 1, fig. 1). The extracellular incorporation at this time is as heavy as, or even heavier than, in the cells. Labelled ground substance is frequently observed in regions where the cells are unlabelled. In the cells sulphate is taken up by the cytoplasm and the nucleus is almost completely negative. Within the cytoplasm there is no specific concentration, either in the perinuclear region or towards the periphery of the cell. The intensity of the autoradiographs varies at the different sites. It is of about the same strength in the cornea and the dorsal skin, but definitely weaker in the pinna, both on the inner and outer sides. After 6 hr. the concentration in the cells in all three sites is weaker, while that of the ground substance and the fibres changes less. The relative differences in intensity between the three sites are as before. Cellular autoradiographs are very weak indeed in all three sites after 16 hr. The extracellular autoradiographs of the connective tissue have decreased in the pinna, remain unchanged in the dorsal skin and have increased in the cornea. After 24 hr. and later the cellular autoradiographs are exceedingly weak, if present at all.

Over the fibrous regions of the connective tissue the autoradiograph after 24 hr. is stronger in the cornea and weaker in the dorsal skin and pinna than before. Four days after the injection of labelled sulphate the extracellular autoradiograph in the substantia propria corneae is increased, while the weak autoradiograph in the pinna remains virtually unchanged and that of the dorsal skin decreases. Pl. 1, figs. 2-4, show the relative strength of the autoradiographs in the three sites.

The illustrations are strictly comparable as the material comes from the same animal, was handled in the same way and the sections were mounted on the same slide for autoradiography. It will be appreciated that the films containing the developed silver grains (i.e. the autoradiograph) are at different levels from the section, which makes it impossible to have the histological detail and the autoradiograph in the same focal plane in a high-power microphotograph. After 6 days there is a further decrease in the autoradiograph over the dorsal skin, but only a slight, if any, decrease in that of the cornea. After 10 days even the extracellular regions of the dermis of the dorsal skin give only a very weak autoradiograph and little or none by the 11th day. In the cornea the autoradiograph remains almost unchanged on the 10th day and is reduced on the 11th day when it equals the peak value for the dorsal skin (observed at 16 hr.), while a further decrease of the autoradiograph of the cornea on the 17th day reduces it to the peak value seen in the pinna after about 2-6 hr.

Table 1. *Relative intensity of the autoradiograph over the extracellular regions of the connective tissue of the cornea, dorsal skin and pinna at different intervals after injection of $^{35}\text{SO}_4$*

Time	Cornea	Dorsal skin	Pinna
2 hr.	+++	+++	++
6 hr.	+++	+++	++
16 hr.	+++	+++	+
24 hr.	+++	++	+
4 days	++++	++	+
6 days	+++ (+)	+	+
10 days	+++ (+)	?	?
11 days	+++	0	0
17 days	++	0	0

Table 1 gives a rough estimate of the intensity of the autoradiograph in the extracellular regions of the connective tissue at the three sites. These estimates are based on the independent assessments of two of the authors whose full agreement indicates that the differences are marked. Some sample counts of grains confirm these estimates. They indicate that in the pinna the smallest amount of labelled sulphate is taken up, the peak value is quickly reached and the labelled sulphate compounds are retained for the shortest period. In the dorsal skin the peak value is also attained quickly, but more labelled sulphate per volume of connective tissue is taken up and retained for considerably longer periods. In the cornea the peak value is reached more slowly, i.e. after 4 days much more labelled sulphate is taken up and retained for a longer time.

Histologically, the connective tissue differs at the three sites in the amount and arrangement of collagen fibre bundles. Thus the corneal tissue is arranged in parallel lamellae and is devoid of vessels. The dorsal skin has a mesh of collagen fibre bundles of fair thickness; a similar arrangement is seen in the outer surface of the pinna while there is less connective tissue on the inner side. The fibre bundles at the inner surface of the pinna may also be slightly thinner than at the outer and in the dorsal skin.

There is no striking difference in the quantitative relation of amount of fibre bundles to connective tissue cells, at least in the pinna and the dorsal skin. No significant difference is found in the staining of the fibre bundles with azan, tolui-

dine blue or with silver stains. Lillie's PAS stain gives a much stronger reaction in the cornea than in the other two sites, and thus parallels the greater uptake of labelled sulphate observed in the autoradiograph.

WOUND-HEALING EXPERIMENTS

The healing of a superficial skin wound begins with the laying down of a fibrinous clot, the surface of which condenses to form the scab, below which repair takes place. A zone of round cells soon demarcates the dense superficial part from the lower part of the clot which, after a latent period, is invaded by capillaries and cells from the sides and from below to form the granulation tissue. The number of fibroblasts increases and fibre formation begins; the fibres are orientated at right angles to the surface and along the vessels until the wound area is covered by the ingrowth of epithelium, after which the fibres are formed in planes parallel to the surface. After the epithelial closure of the wound the scab is shed. Ingrowth of epithelium and fibre formation, together with contraction of the wound, are responsible for the production of the scar.

In our experiments the animals were injected intraperitoneally with $\text{Na}_2^{35}\text{SO}_4$ 24 hr. before they were killed, and the autoradiographs thus show the uptake and retention of sulphate over a 24 hr. period in different stages of wound-healing. The following points were especially investigated: (1) the relation of the fibrinous clot to fibre formation; (2) the uptake of sulphate by fibroblasts before and during fibre formation; (3) the uptake of sulphate by extracellular material in various stages of fibre formation; and (4) the possible role of mast cells in fibre formation.

The superficial scab, down to the zone of demarcation by round cells, stains strongly with Lillie's PAS, but it is not metachromatic with toluidine blue nor does it show any significant uptake of labelled sulphate at any stage of healing. After 2 days the clot below the zone of demarcation contains metachromatic material and stains less intensely with PAS than the scab; from this time onwards it also shows a slight uptake of labelled sulphate (Pl. 1, fig. 5). As the fibrinous material is invaded by macrophages and later by capillaries and fibroblasts, it is broken up into larger diffuse masses and into smaller denser strands which stain more intensely with PAS. Both the strands and the more diffuse masses show a slight uptake of labelled sulphate. The invading macrophages contain PAS-positive granules and some basophilic material, but little, if any, labelled sulphate.

The fibroblasts accumulate at the edges of the wound and perivascularly, multiply and penetrate the metachromatic material of the clot by about the 5th day. These cells take up a considerable amount of labelled sulphate in their cytoplasm (Pl. 1, fig. 6). As fibres are laid down, labelled sulphate appears outside the cells (Pl. 1, fig. 7) in the newly formed fibres, while the uptake by the cells decreases. The fibre bundles take up labelled sulphate very strongly throughout the 18 days of the observation period.

Stages in fibre formation are seen in any given wound as this process progresses from the periphery to the centre. From the 5th day onward, under the conditions of our experiments, at first the fibroblasts and later the fibres retain the injected labelled sulphate. Neither the fibroblasts nor the fibres give the reddish metachromatic stain with toluidine blue which appears in the fibrinous clot and strongly

also in the mast cells after the fixation used for our material. The newly formed fibres give a strong stain with PAS, and some PAS granules, as well as basophilic material, are also found in the fibroblasts.

Mast cells give a strong autoradiograph. They are found at first only outside the wound area and do not appear in the wound region until after the immigration of fibroblasts and the beginning of fibre formation. Though the concentration per cell of labelled sulphate in mast cells is far greater than in fibroblasts, this difference does not compensate for the difference in number of mast cells and fibroblasts. During the initial process of fibre formation the mast cells are too few and too far removed from the scene of collagen formation to contribute materially to the amount of compounds containing sulphur which appear in the fibrous material.

Our observations suggest that the fibrinous clot is not directly utilized in the formation of fibres, that fibroblasts take up labelled sulphate markedly before and during fibre formation and less markedly in later stages of fibre formation; that labelled sulphate is also taken up by fibres which are still growing and that there is no evidence for a material contribution of sulphated compounds by the mast cells to the initial stages of fibre formation.

DISCUSSION

Bostrom & Jorpes (1954) have shown that after intraperitoneal injection, labelled inorganic sulphate rapidly disappears from the bloodstream whilst it is incorporated into various substances such as chondroitin sulphate which only slowly loses its activity. These authors suggest that ^{35}S is incorporated into the sulphated mucopolysaccharides of, for instance, the cartilage, cornea, sclera and mast cells. It seems reasonable to assume that the autoradiographs obtained over the cells and fibres of the connective tissue in our experiments are due to the incorporation of labelled sulphate into various mucopolysaccharides. In favour of this suggestion is the fact that the structures giving an autoradiograph also stain with PAS (Leblond, 1950; Belanger, 1954).

We have no means of deciding whether the incorporation of labelled sulphate into various mucopolysaccharides at different sites (Meyer, 1950; Kent & Whitehouse, 1955) accounts for the observed difference in uptake as regards both amount and rate between the connective tissues of the dorsal skin, pinna and cornea (cf. Table 1). The difference between the cornea and the two skin sites might be due to the presence of different mucopolysaccharides or to the difference in vascularization. As the amount of labelled sulphate taken up initially by the cornea equals that of the dorsal skin and exceeds that of the pinna, it seems that the amount of labelled sulphate taken up is not primarily dependent on vascular supply. The different behaviour of the cornea as regards the uptake of labelled sulphate might be due to the known presence of three distinct mucopolysaccharides in this structure, which may differ in their metabolism: a chondroitin sulphate, a sulphated hyaluronic acid and the keratosulphate (Kent & Whitehouse, 1955). One of the possible explanations is that one of the mucopolysaccharides of the cornea (the keratosulphate), which is not present in the skin, takes up labelled sulphate later and retains it longer, thus increasing the total uptake of labelled sulphate and delaying both the appearance of the peak and the subsequent disappearance of the isotope.

Even mature connective tissue has an appreciable maintenance metabolism of sulphated compounds; this metabolism occurs partly, if not mainly, outside the cells and differs at different sites. This uptake in mature connective tissue is, however, much lower than that in regenerating connective tissue of wounds where the cells play a significant role in the initial stages. There is a comparable difference in the uptake of labelled sulphate between granulation tissue of muscle wounds and intact muscles (Layton, 1951) and between regenerating and mature tendon (Kodicek & Loewi, 1955), between young and old cartilage (Amprino, 1954, 1955 *a, b*; Friberg & Ringertz, 1954) and in the uptake of phosphate between young and old osteons of bone (Engfeldt, Engstrom & Zetterstrom, 1952). For the mature cartilage we have previously shown (Pelc & Glücksmann, 1955) that the labelled sulphate is taken up primarily by the chondrocytes and only slowly released into the ground substance. In this respect the cartilage behaves differently from the mature connective tissue which takes up the labelled sulphate mainly extracellularly.

Since in the fibrous connective tissue the ^{35}S uptake is mainly extracellular and since the collagen fibrils are protein, it is plausible to assume that the labelled sulphate is situated in the interfibrillar ground substance, which may undergo continuous change (Gersh, 1951). What role the presumed sulphated mucopolysaccharides play in the structure and function of the ground substance, remains obscure. It is tempting to think of them as forming the apparently amorphous and hyaluronidase-resistant background for the minute membranes of fibrils seen in electronmicrographs by Day & Eaves (1953).

Meyer (1950) suggests that fibroblasts secrete an acid mucopolysaccharide, together with a globular protein, and that by precipitation of a precollagen on to the mucopolysaccharide they form the reticulin fibres; the mucopolysaccharides first surround the fibrils and are later digested by enzymes. This concept would account for the much heavier uptake of sulphate, first by fibroblasts and then by the newly formed fibres in the regenerating connective tissue as compared with the mature connective tissue. The metachromatic material in the fibrinous clot takes up only little sulphate, is susceptible to the action of hyaluronidase (Campani & Reggianini, 1950), and is replaced by the vascular and cellular granulation tissue; it does not appear to contribute to the sulphated compounds found in the newly formed fibrous tissue which is not susceptible to the action of hyaluronidase (Campani & Reggianini, 1950; Curran, 1953). Similarly, the mast cells do not contribute sulphated compounds to the formation of such compounds in regenerating connective tissue. Whether they later secrete a non-sulphated substance such as hyaluronic acid (Asboe-Hansen, 1954) into the connective tissue cannot be decided from our experiments.

It is interesting that the fibroblasts and newly formed fibrous tissue take up appreciable amounts of sulphate, and that at the same stages they are also found to be highly phosphatase-positive (Fell & Danielli, 1943; Kodicek, 1951). At the same time the PAS-staining of the fibres is also most intense. While there is some general correlation between PAS-staining, metachromatic staining with toluidine blue and sulphate uptake (Belanger, 1954), the correlation is not absolute. This is not surprising since the intensity of histological or histochemical stains depends on the concentration of any given substance, while the autoradiograph method shows

the newly synthesized or re-formed part of that compound. It is of value in thus demonstrating the existence of a continuous change, even in the ground substance of mature connective tissue and of differences in the rate of this change at different sites.

SUMMARY

1. The uptake, retention and loss of labelled sulphate in the connective tissue of the pinna, dorsal skin and cornea were studied in autoradiographs made at intervals ranging from 2 hr. to 17 days after the intraperitoneal injection of labelled sulphate into adult mice.

2. In mature connective tissue labelled sulphate is taken up by cells and fibres. The cellular uptake is relatively weak and of short duration, while more sulphate is taken up extracellularly and retained for a longer time.

3. The rate and amount of extracellular sulphate uptake in mature connective tissue varies with site: in the pinna little sulphate is taken up and retained for a short time; in the dorsal skin more sulphate is taken up and retained longer; in the cornea considerably more sulphate is taken up and retained considerably longer than in the other two sites. The peak of extracellular sulphate uptake is reached after 2–6 hr. in the pinna, after 6–16 hr. in the dorsal skin and after 4 days in the cornea.

4. Standard skin wounds were produced, and the uptake of labelled sulphate studied in autoradiographs made 24 hr. after the intraperitoneal injection of sulphate given at intervals of 1 to 18 days after producing the wound.

5. The fibrinous clot and scab of the wound take up little, if any, sulphate. The fibroblasts of the granulation tissue give a strong autoradiograph before and during fibre formation and so do the newly formed and growing fibres. The intracellular precedes the extracellular sulphate uptake in the growing and newly formed connective tissue.

6. The total sulphate uptake is far greater in the developing than the mature connective tissue.

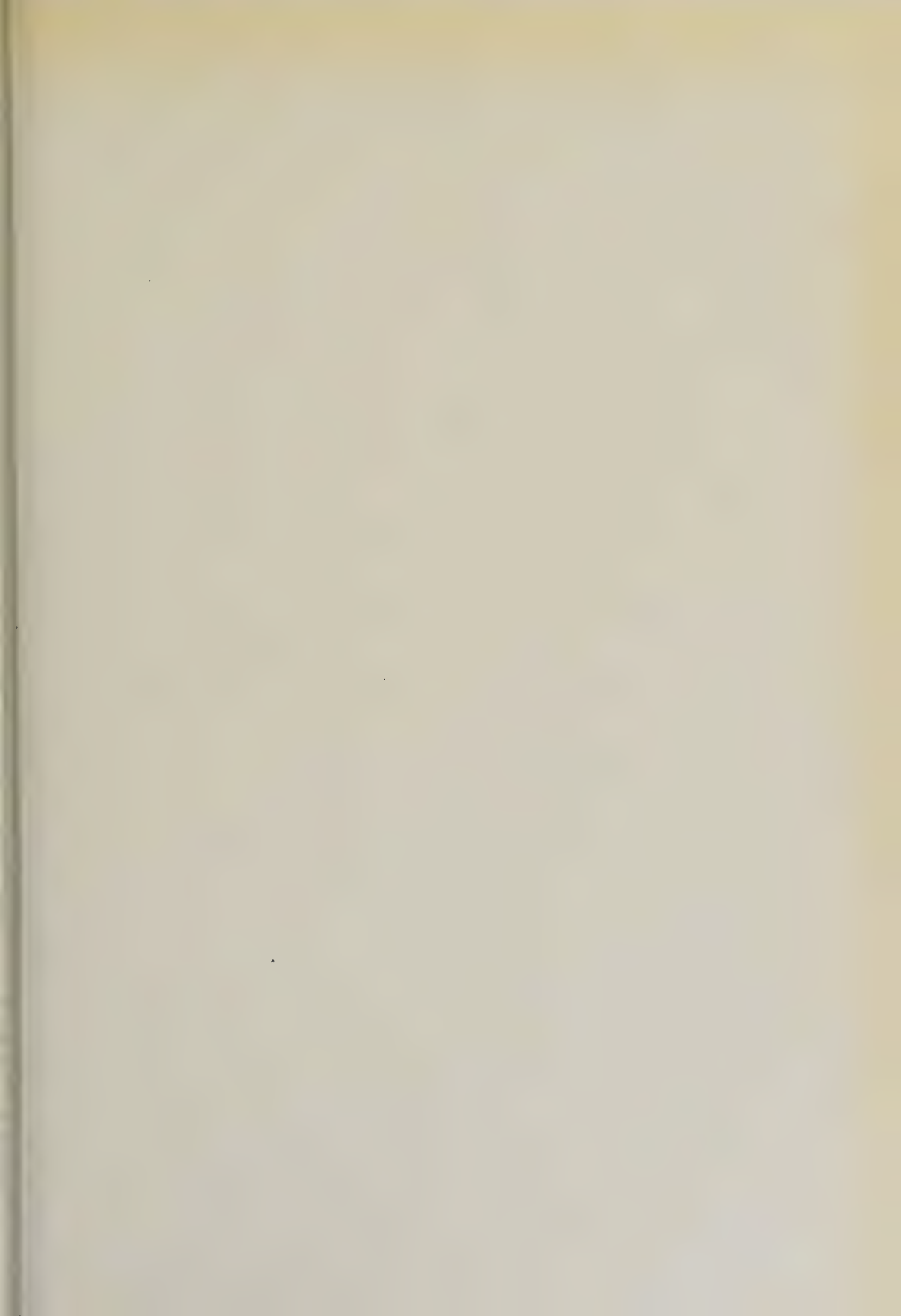
7. There is no evidence that the fibrinous clot or the mast cells contribute to the formation of sulphur-containing compounds of the connective tissue.

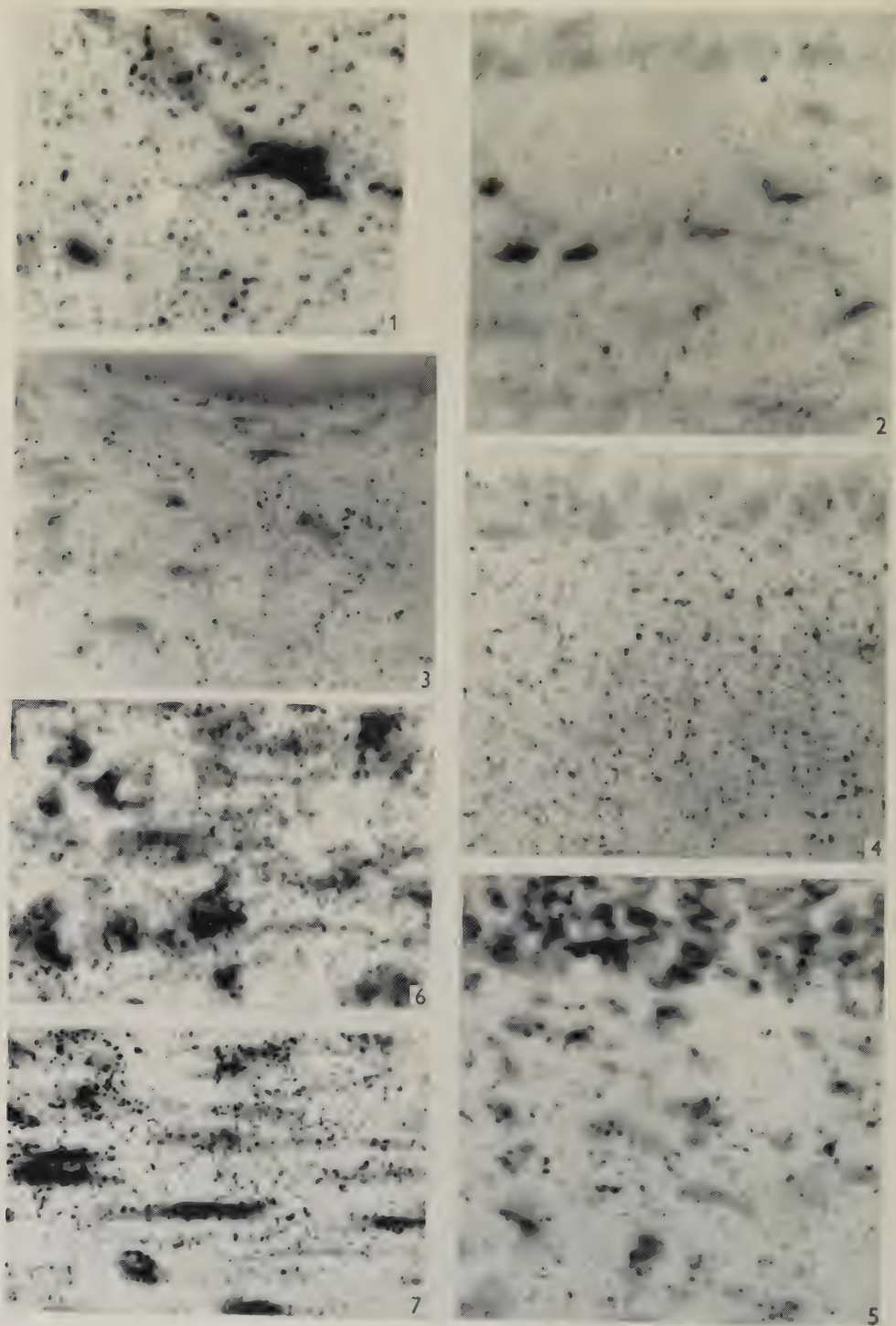
8. The findings are discussed in relation to the presence and metabolism of mucopolysaccharides in mature and developing connective tissue.

We wish to record our thanks to Dr H. B. Fell, F.R.S., and to Dr G. Popjak for helpful criticisms of the manuscript, and to Mr G. C. Lenney for the photomicrographs.

REFERENCES

- AMPRINO, R. (1954). Differenziazione e accrescimento della cartilagine di abbozzi scheletrici studiati con l'impiego del radiosolfo. *R.C. Accad. Lincei*, s. VIII, **16**, 781–785.
- AMPRINO, R. (1955*a*). On the incorporation of radiosulfate in the cartilage. *Experientia*, **11**, 65–67.
- AMPRINO, R. (1955*b*). Distribution of ^{35}S -sodium sulfate in early chick embryos. *Experientia*, **11**, 19–20.
- ASBOE-HANSEN, G. (1954). The mast cell. *Int. Rev. Cytology*, **3**, 399–435.
- BELANGER, L. F. (1954). Autoradiographic visualization of ^{35}S incorporation and turnover by the mucous glands of the gastrointestinal tract and other soft tissues of rat and hamster. *Anat. Rec.* **118**, 755–772.
- BOSTROM, H. & JORPES, E. (1954). On the enzymatic exchange of the sulphate group of the animal sulpho-mucopolysaccharides. *Experientia*, **10**, 392–396.





GLÜCKSMANN, HOWARD AND PELC—UPTAKE OF LABELLED SULPHATE BY CONNECTIVE TISSUE IN THE MOUSE

(Facing p. 485)

- CAMPANI, M. & REGGIANINI, O. (1950). Observations in the experimental animal on the nature of the metachromatic ground substance in granulation tissue. *J. Path. Bact.* **62**, 563-568.
- CAMPBELL, D. & PERSSON, B. H. (1951). Use of track autoradiography in studies on the sulfur metabolism of connective tissue. *Experientia*, **7**, 304-309.
- CURRAN, R. C. (1953). Observations on the formation of collagen in quartz lesions. *J. Path. Bact.* **66**, 271-282.
- DANN, L., GLÜCKSMANN, A. & TANSLEY, K. (1941). The healing of untreated experimental wounds. *Brit. J. exp. Path.* **22**, 1-8.
- DANN, L., GLÜCKSMANN, A. & TANSLEY, K. (1942). Experimental wounds treated with cod-liver oil and related substances. *Lancet*, **1**, 95-97.
- DAVIES, D. V. & YOUNG, L. (1954). The distribution of radioactive sulphur (^{35}S) in the fibrous tissues, cartilages and bones of the rat following its administration in the form of inorganic sulphate. *J. Anat., Lond.*, **88**, 174-183.
- DAY, T. D. & EAVES, G. (1953). Electron microscope observations of the ground substance of interstitial connective tissue. *Biochim. biophys. acta*, **10**, 203-209.
- DONIACH, I. & PELC, S. R. (1950). Autoradiograph technique. *Brit. J. Radiol.* **23**, 184-192.
- ENGELFELDT, B., ENGSTROM, A. & ZETTERSTROM, R. (1952). Renewal of phosphate in bone minerals. *Biochim. biophys. acta*, **8**, 375-380.
- FELL, H. B. & DANIELLI, J. F. (1943). The enzymes of healing wounds. *Brit. J. exp. Path.* **24**, 196-203.
- FRIBERG, U. & RINGERTZ, N. R. (1954). Autoradiographic studies with ^{35}S on the development of the rat embryo. *Experientia*, **10**, 67-68.
- GERSH, I. (1951). Some functional considerations of ground substance of connective tissues. *J. Macy Found. 2nd Conf. on Connective Tissues*, pp. 11-44.
- KENT, P. W. & WHITEHOUSE, M. W. (1955). *Biochemistry of the Aminosugars*. London: Butterworth.
- KODICEK, M. E. (1951). Joints, bones and wound healing in partial Vitamin C deficiency in guinea pigs. *Trans. of 5th Congr. Soc. Int. Chir. Orthoped. Traumatol., Stockholm*, pp. 424-439.
- KODICEK, M. E. & LOEWI, G. (1955). The uptake of (^{35}S) sulphate by mucopolysaccharides of granulation tissue. *Proc. Roy. Soc. B*, **144**, 100-115.
- LAYTON, L. L. (1951). The anabolic metabolism of radioactive sulfate by animal tissues *in vitro* and *in vivo*. *Cancer*, **4**, 198-201.
- LEBLOND, G. P. (1950). Distribution of periodic acid-reactive carbohydrates in the adult rat. *Amer. J. Anat.* **86**, 1-50.
- MEYER, K. (1950). *J. Macy Found. 1st Conf. on Connective Tissues*, p. 32.
- PELC, S. R. & GLÜCKSMANN, A. (1955). Sulphate metabolism in the cartilage of the trachea, pinna and xiphoid process of the adult mouse as indicated by autoradiographs. *Exp. Cell. Res.* **8**, 336-344.

EXPLANATION OF PLATE

- Fig. 1. Autoradiograph of dermal tissue in the dorsal region 2 hr. after intraperitoneal injection of labelled sulphate. The cells of the fibrous tissue are stained with carmalum-neutral red while the fibres are unstained. Note the marked extracellular and the slight cellular uptake of sulphate. $\times 1000$.
- Figs. 2-4. Autoradiographs of the connective tissue of the pinna (fig. 2), dorsal skin (fig. 3) and cornea (fig. 4) 4 days after intraperitoneal injection of labelled sulphate. Note the difference in the intensity of the mainly extracellular autoradiographs which are strongest in the cornea and weakest in the pinna. Carmalum-neutral red. $\times 680$.
- Fig. 5. Autoradiograph of the fibrinous clot in a 2-day-old wound in an adult mouse 24 hr. after intraperitoneal injection of labelled sulphate. The zone of demarcating round cells is seen at the top of the picture. Carmalum-neutral red. $\times 680$.
- Fig. 6. Autoradiographs of the granulation tissue in a 5-day-old wound in an adult mouse 24 hr. after intraperitoneal injection of labelled sulphate. Uptake is seen mainly in the cytoplasm of the fibroblasts. Carmalum-neutral red. $\times 680$.
- Fig. 7. Autoradiograph of the granulation tissue in a 7-day-old wound in an adult mouse 24 hr. after intraperitoneal injection of labelled sulphate. Heavy uptake is seen in the fibroblasts and extracellularly in the newly formed fibres. Carmalum-neutral red. $\times 680$.

THE BLOOD SUPPLY OF THE OPTIC NERVE AND CHIASMA IN MAN

BY E. J. STEELE AND M. J. BLUNT*

*Department of Anatomy, Royal Free Hospital
School of Medicine*

Though numerous observations have been made on the blood supply of the optic nerve in man and there is general agreement on its gross arrangement, the recorded evidence is conflicting in several important details, particularly touching the part played by the central retinal artery in the nutrition of the nerve.

All authors are agreed that the central retinal artery contributes branches to the pial plexus before entering the nerve. An intraneural distribution of branches of the central retinal artery has been described by some authors (Beauvieux & Ristitch, 1924; Duke-Elder, 1932; Traquair, 1946) and denied by others (François, Neetens & Colette, 1955). The most proximal of the branches distributed intraneurally and said to arise from the central retinal artery in its extraneural course, enters the nerve as a branched or unbranched collateral of the main artery. Branches of the central retinal artery arising in the substance of the optic nerve have also been reported (Duke-Elder, 1932; Abbie, 1938; Wolff, 1940; Bignell, 1952), and some authors have described a direct arterial anastomosis between the most distal of these and branches from the anastomotic circle of posterior ciliary arteries, the circle of Zinn (Duke-Elder, 1932; Sauter & Seitz, 1952). Other authors deny the existence of any intraneural branches (Magitot, 1908, quoted by Poirier & Charpy, 1912; Beauvieux & Ristitch, 1924).

François *et al.* (1955) have described the detailed intrinsic vascular anatomy of the optic nerve. Cristini (1951) has reported on the intrinsic vascular anatomy of forty nerves taken from patients with glaucoma, and his incidental references to the normal vascular pattern do not agree with the findings of the last-named workers.

Abbie (1938) and Dawson (1948) both investigated the arterial supply of the optic chiasma and Xuereb, Pritchard & Daniel (1954) commented on certain features of the supply to the chiasma and optic tract. Dawson alone briefly described the pattern of capillaries in the chiasma.

The potential clinical significance of the blood supply of the optic nerve and chiasma is now increasingly appreciated, as, for example, in the aetiology of glaucoma and in connexion with the surgery of the pituitary gland. It was considered necessary, therefore, to reinvestigate the vascular pattern of the nerve and chiasma, both with a view to resolving the doubts and contradictions apparent in the literature and to clarifying the descriptive vascular anatomy of these structures.

* Present address: Department of Anatomy, Medical College of St Bartholomew's Hospital.

MATERIAL AND METHODS

Fresh human autopsy material was obtained from thirty-six subjects, males and females whose ages ranged from 10 weeks to 88 years and who were free from known ocular disease.

(a) In seventeen subjects the vessels were injected; in fourteen of these injection was made simultaneously into both internal carotid arteries in the neck, and in the remaining three cases directly into each ophthalmic artery *in situ*. Coloured 'Neoprene' was used for ten injections into the internal carotid arteries and for the injections into the ophthalmic arteries, a pressure of 200 mm. of mercury being used. In the remaining four, coloured 'Micropaque' was injected at a pressure of 100 mm. of mercury.

In three subjects the entire contents of the orbit were subsequently removed *en bloc*, and in fourteen the posterior third of the eyeball with the optic nerve was removed with as much surrounding tissue as possible. The chiasma was removed in continuity with the optic nerves in six subjects, and in the remaining eleven the brain, chiasma, pituitary body and surrounding bone were removed together. All excised material was fixed immediately in 10% formol saline and was then dissected under the microscope.

(b) In a second group of seventeen subjects, the optic nerves and chiasmata were removed, fixed in hypertonic formol saline for a minimum of 3 days and embedded in gelatin as described by Blunt (1954). Longitudinal frozen sections, from 200 to 300 μ thick, were cut from the nerves of sixteen of these subjects and transverse sections were cut from the nerves of the remaining subject. Horizontal sections of corresponding thicknesses were cut from sixteen chiasmata and one chiasma was cut sagittally. All sections were treated with sodium nitroprusside and benzidine as described by Pickworth (1934), and then cleared and mounted on ringed slides in Spalteholz's fluid, a convenient medium for mounting thick sections stained by this method (Scott, 1955, personal communication).

The nerves and chiasma from a single subject were fixed in 10% formol saline and embedded in paraffin. Serial sections at 10 μ were cut longitudinally from one nerve and transversely from the other, the chiasma being cut horizontally. Alternate slides were stained with haematoxylin and Biebrich Scarlet and with Mallory's triple stain.

The nerves and chiasma from a subject in which injection had been unsuccessful were embedded in paraffin and sectioned at 80 and 100 μ ; the nerves were sectioned longitudinally and the chiasma horizontally, and the sections stained with van Gieson's stain.

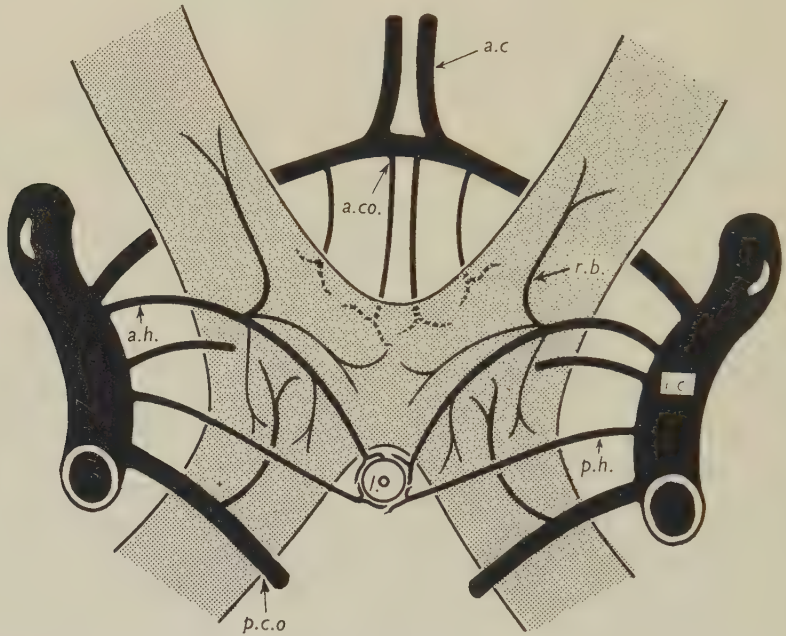
FINDINGS

Blood supply of the chiasma (Text-fig. 1)

The chiasma was, in general, supplied by vessels which ramified in the overlying pial network. On its inferior surface the largest vessel contributing to the network was the anterior superior hypophyseal artery which constantly gave off a recurrent branch along the infero-medial border of the proximal part of the optic nerve. The internal carotid and posterior communicating arteries also supplied the pial plexus (Pl. 1, fig. 1). The posterior superior hypophyseal artery gave no branches to the

chiasma in this series. On the superior surface of the chiasma the pial plexus received vessels from the anterior cerebral and anterior communicating arteries (Pl. 1, fig. 2).

Venous networks along the antero-inferior border of the chiasma were connected by transverse branches and they drained into the basal veins; those from the superior surface of the chiasma drained into the anterior cerebral veins. These chiasmal veins were clearly visible to the naked eye although, as expected, they were not filled with the injection masses used.



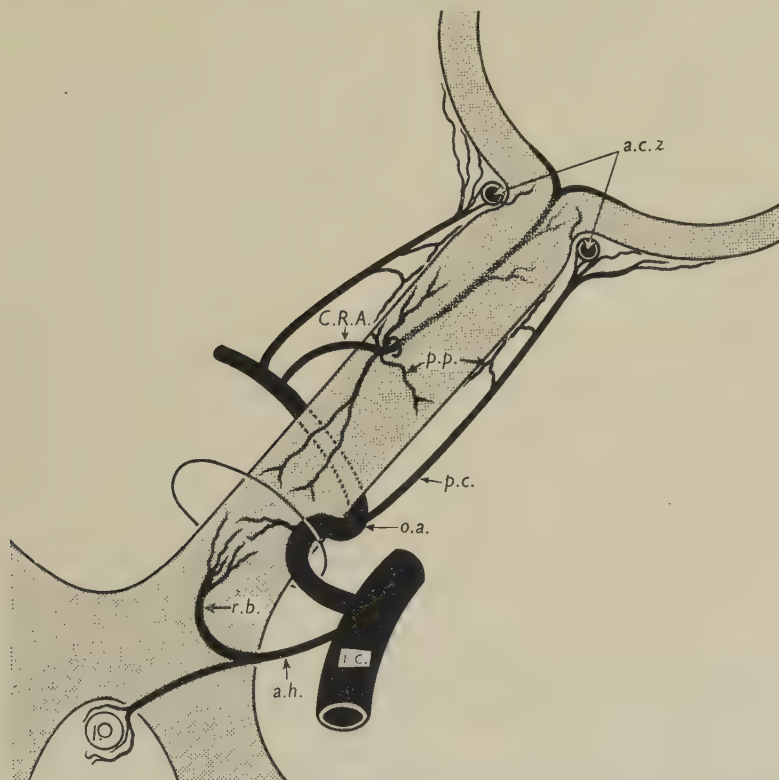
Text-fig. 1. The arterial blood supply of the optic chiasma.

In all sections of the seventeen chiasmata stained by the sodium nitro-prusside-benzidine technique the intrinsic capillary plexus was seen to be connected with vessels in the overlying pial network. The capillary vessels within the chiasma crossed from side to side and anastomosed freely in the mid-line. Vessels within the medial part of the optic nerve were continuous with those within the anterior part of the chiasma, but the intrinsic vessels from the lateral part of the nerve tended to course within the lateral margin of the chiasma into the corresponding optic tract. Posteriorly the capillaries of the optic chiasma were directly continuous with those of the hypothalamus (Pl. 2, fig. 6). The glial septa in the chiasma were sparse and irregular, and their arrangement bore no resemblance to that of the capillaries.

Blood supply of the optic nerve (Text-fig. 2)

For convenience, details of the arterial supply of the nerve will be described separately for its intracranial, intracanalicular and intraorbital parts. Like the chiasma all three parts of the nerve were supplied through a continuous overlying pial plexus.

The intracranial part of the nerve was constantly supplied by the recurrent branch of the anterior superior hypophyseal artery (Pl. 1, fig. 1) and by small recurrent branches of the ophthalmic artery which joined the pial network. The intracanalicular part of the nerve received its blood supply from small branches of the ophthalmic artery given off just distal to the optic canal (Pl. 1, fig. 4).



Text-fig. 2. Diagrammatic representation of findings on the blood supply to the optic nerve.
Left side, seen from below.

The pial plexus of the intraorbital part of the nerve received branches from the posterior ciliary arteries throughout its course. The central retinal artery was seen to give branches to the pial network close to its entry into the nerve (Pl. 1, fig. 4) in fifteen out of seventeen nerves in which injection was satisfactory up to this point.

The central retinal artery arose from the ophthalmic in twenty-eight out of thirty dissections and from a posterior ciliary artery in two. It entered the infero-medial surface of the nerve at a distance varying from 7 to 17 mm. behind the optic disc. In all of nine nerves in which the central retinal artery was fully injected, intraneural branches were found. In seven of these a single branch left the artery midway along its intraneural course and in the other two nerves two such intraneural branches were given off (Pl. 1, fig. 5). This intraneural branching of the artery was confirmed in transverse sections of the nerves (Pl. 2, fig. 9). In no instance were any intraneurally distributed direct branches of the central retinal artery observed in the region of the lamina cribrosa, neither was any cilio-retinal artery seen.

The intrinsic and extrinsic veins draining the optic nerve were readily visible though unfilled by the injection masses, the central retinal vein regularly accompanying its companion artery. The former left the nerve just posterior to the point of entrance of the artery. In both longitudinal and transverse sections, the vein was seen to receive numerous tributaries throughout its intraneural course (Pl. 1, fig. 3; Pl. 2, fig. 9).

In all the nerves stained by the sodium nitroprusside-benzidine technique constant regional variations were found in the pattern of the intrinsic capillary plexus. In the intracranial part of the nerve the capillaries were found to be irregularly disposed and to exhibit frequent spiral formations (Pl. 2, fig. 8). Thick sections stained by van Gieson's method demonstrated a remarkable correspondence between the arrangement of the glial septa and these vessels. In both the intracanalicular and the intraorbital parts of the nerve, the capillaries were arranged in a definite box pattern (Pl. 2, fig. 10) which also corresponded to the local arrangement of the glial septa (Pl. 2, fig. 7).

In the region of the lamina cribrosa small branches from the posterior ciliary arteries entered the pial plexus and choroid (Pl. 1, fig. 3). The largest of these vessels were of not more than pre-capillary size. Examination of all sections failed to reveal any evidence of the existence of direct arterial anastomoses between branches of the central retinal artery and branches from the anastomotic circle of posterior ciliary arteries. The capillaries in the lamina cribrosa were closely packed (Pl. 1, fig. 3) and were in direct continuity anteriorly with those of the choroid and retina and posteriorly with those of the retrolaminar portion of the optic nerve.

DISCUSSION

As is well known, the results of most injection techniques are variable, and more reliance can be placed upon positive than upon purely negative findings. In the present investigation conclusions have been drawn only from preparations in which there was evidence of an injection sufficiently complete to justify their inclusion in the series. The results of sodium nitroprusside-benzidine staining have proved remarkably constant, so that the findings reported here have been observed in every relevant specimen. They provide valuable confirmatory evidence of the results obtained from injection methods.

The findings on the gross blood supply of the optic chiasma accorded with those of Dawson (1948). The main features of interest, and of possible practical significance, were that the largest, most numerous and most extensive branches to the pial network on the inferior surface of the chiasma and intracranial part of the optic nerve were regularly supplied by the anterior superior hypophyseal artery. The pial network on the inferior surface did, however, provide a means of free intercommunication between adjacent arteries of supply. The capillary vessels of the intrinsic plexus tended in general to follow the course of the nerve fibres through the chiasma rather than to follow the disposition of the glial septa. The free intercommunication of the intrinsic capillary vessels of the chiasma with those of the hypothalamus was most striking.

The present investigation has shown that the main supply of the intracranial part of the optic nerve was dependent upon the recurrent branch of the anterior superior

hypophyseal artery with a few additional twigs from the ophthalmic artery. Dawson (1948) attributed greater importance to these latter branches, regarding them as the main supply of the intracranial portion of the nerve. Though the total number of his specimens was larger, the findings in the present more limited series were invariable. Wolff's (1954) statement that the internal carotid, anterior cerebral and anterior communicating arteries played a part in the supply of this region of the nerve received no confirmation from the present investigation; his observation that the intracanalicular part of the nerve was supplied by the ophthalmic artery was, however, confirmed.

The present work also confirmed previous statements regarding the arterial supply to the pial plexus (Poirier & Charpy, 1912; Duke-Elder, 1932; Wolff, 1939). In fifteen specimens in which the extraneural pial branches were filled there was no evidence of an extraneural branch of the central retinal artery accompanying it into the nerve (Beauvieux and Ristitch, 1924; Duke-Elder, 1932; Traquair, 1946). In nine of these specimens the central retinal artery was completely filled throughout its course, thus indicating that any collateral branch is far from constant. On the other hand, there was unequivocal support for those (Duke-Elder, 1932; Abbie, 1938; Wolff, 1940; Bignall, 1952) who described branches of the central retinal artery in the substance of the optic nerve. In no instance, either in adequately injected specimens or in sodium nitroprusside-benzidine preparations, was there any evidence of a direct arterial anastomosis between the central retinal artery and vessels derived from the circle of Zinn.

A cilio-retinal artery, a common feature of certain mammals including Carnivora, Marsupials and some Ungulates (Nettleship, 1905; Duke-Elder, 1932), is sometimes found in man. Its incidence, as estimated from the results of ophthalmoscopic examination, is variously given as 16.7% of forty-eight eyes by Lang & Barrett (1889), 14.2% of 120 eyes by Veasey, 8.7% of 439 eyes by Yoshida (Adachi, 1928), and 'at least 25%' by Mann (1937). No evidence of this vessel has, however, been found in the present investigation.

François *et al.* (1955) used micro-arteriography as a means of investigation of the blood supply to the optic nerve. Although this difference in technique should not make it impossible to compare results, it is nevertheless difficult to do so. They stated that the capillary network was 'not so rich' as the lamina cribrosa was approached, and 'towards the papilla at the region of the physiological excavation of the cup they disappear', and later in the same paper that 'the vascularization of the nerve becomes gradually less dense from the papilla to the optic foramen'. In the light of these apparently irreconcilable statements it is difficult to interpret their findings.

Although in this present investigation no direct arterial anastomosis has been found between branches of the central retinal artery and those from the anastomotic circle of Zinn, the dense capillary plexus in the region of the lamina cribrosa was noted to be in direct continuity with the choroidal capillary vessels. This suggests that under conditions of raised intra-ocular tension, the choroidal capillaries connected with those of the lamina cribrosa might possibly be compressed against the spur of sclera which projects into the nerve head (Pl. 1, fig. 3), thus impairing the nutrition of the nerve fibres in the lamina.

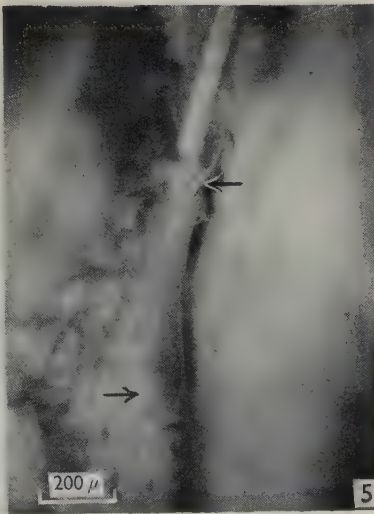
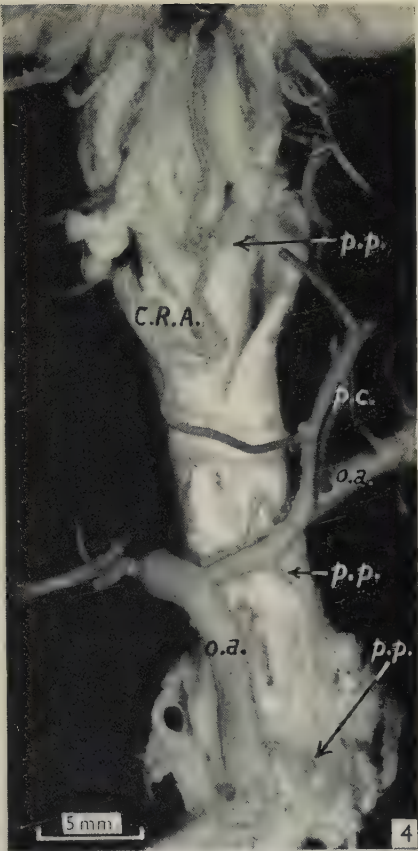
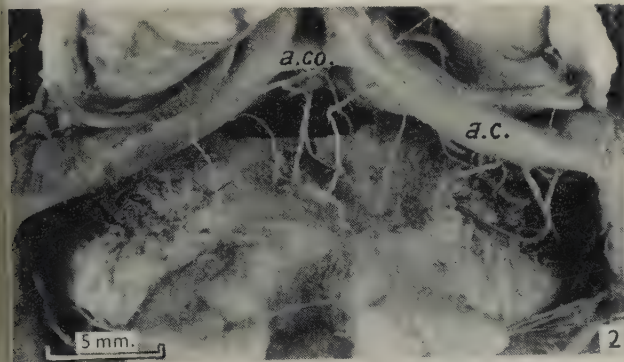
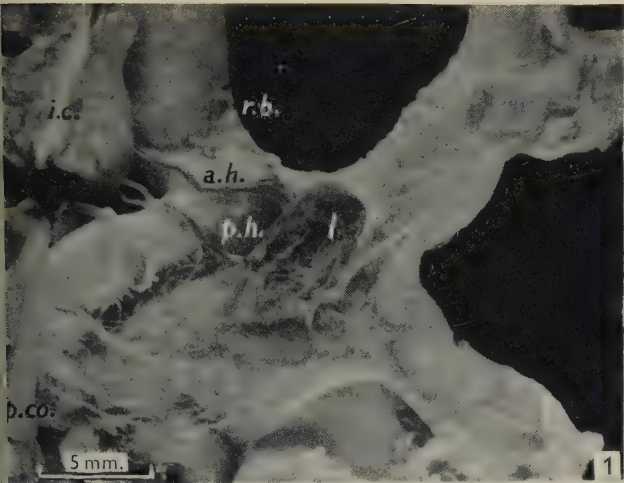
SUMMARY

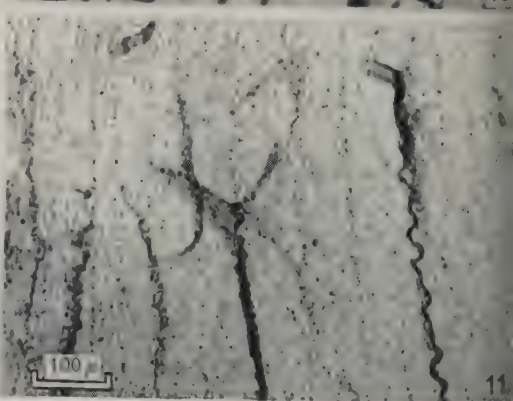
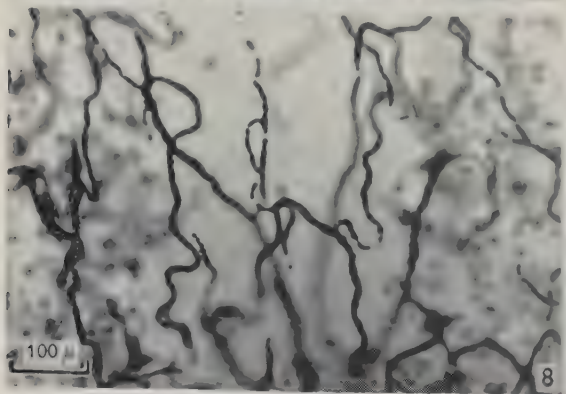
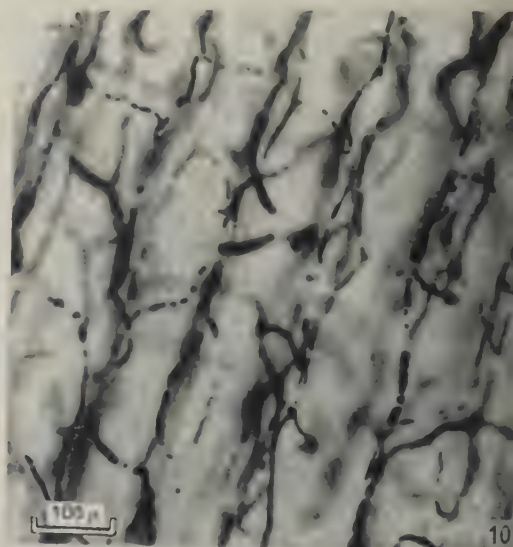
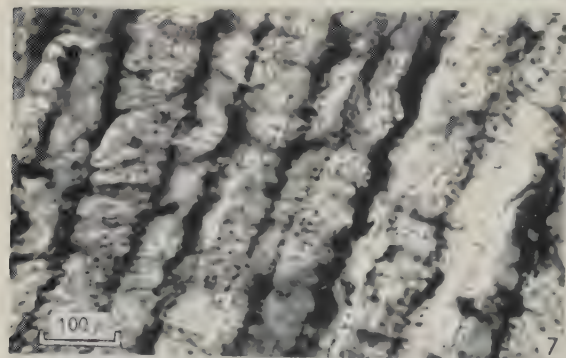
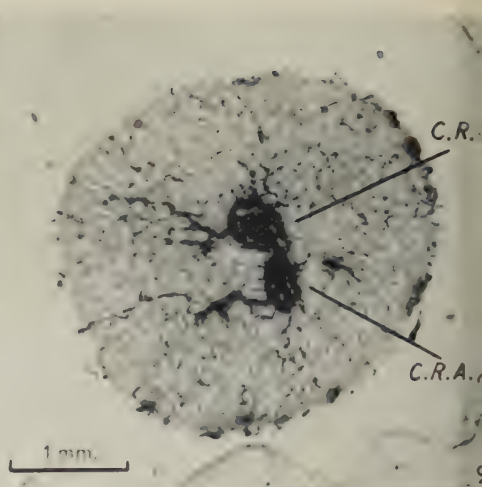
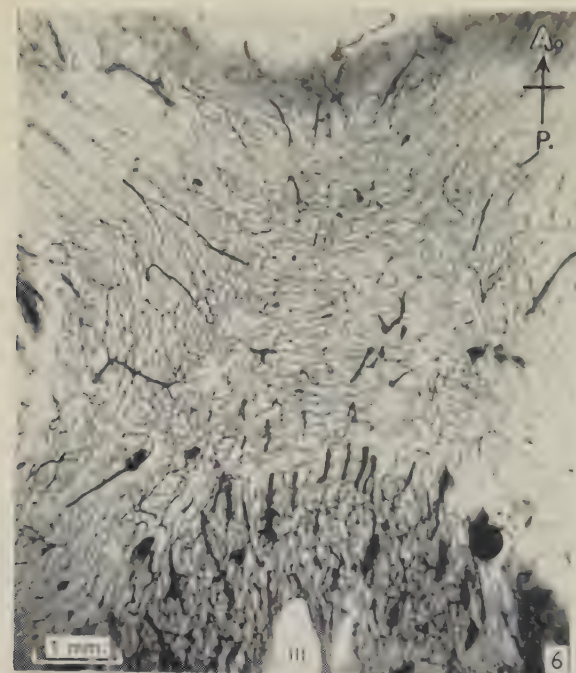
The gross blood supply and the intrinsic vascular anatomy of the optic nerve and chiasma have been described and the findings discussed in relation to previously reported observations; the significance of some of the results has been suggested.

The authors are grateful to Prof. R. E. M. Bowden for her constant advice and encouragement and helpful criticism of the manuscript, to Prof. A. J. E. Cave who has also kindly criticized the manuscript, and to Dr Francis Camps and his staff who provided the material for the investigation. Valuable technical assistance was provided by Mr L. V. Grayson and Mr W. Matthews. The photographs are the work of Mrs J. M. Thomas and the text-figures that of Mrs A. Besterman. The Augustus and Alice Waller Research Trust provided part of the photographic apparatus. One of us (E.J.S.) is grateful to the Council of the Royal Free Hospital School of Medicine for help afforded by the award of the Julia Ann Hornblower Cock Scholarship and the Mabel Webb and A. M. Bird Research Scholarship.

REFERENCES

- ABBIE, A. A. (1938). Blood supply of the visual pathways. *Med. J. Aust.* **2**, 199-202.
- ADACHI, B. (1928). *Das Arteriensystem der Japaner*, **1**, 109-110. Kyoto.
- BEAUVIEUX, J. & RISTITCH, K. (1924). Les vaisseaux centraux du nerf optique. Étude anatomique. *Arch. ophthal., Paris*, **41**, 352-369.
- BIGNELL, J. L. (1952). Investigations into the blood supply of the optic nerve with special reference to the lamina cribrosa region. *Trans. ophthal. Soc. Aust.* **12**, 150-158.
- BLUNT, M. J. (1954). The blood supply of the facial nerve. *J. Anat., Lond.*, **88**, 520-526.
- CRISTINI, G. (1951). Common pathological basis of the nervous ocular symptoms in chronic glaucoma. *Brit. J. Ophthal.* **35**, 11-20.
- DAWSON, B. H. (1948). *On the blood vessels of the human optic chiasma, hypophysis and hypothalamus*. M.D. Thesis, Manchester.
- DUKE-ELDER, S. (1932). *Textbook of Ophthalmology*, vol. i. London: Kimpton.
- FRANÇOIS, J., NEETENS, A. & COLETTE, J. M. (1955). Vascular supply of the optic pathway. II. Further studies by microarteriography of the optic nerve. *Brit. J. Ophthal.* **39**, 220-232.
- LANG, W. & BARRETT, J. W. (1889). On the frequency of cilio-retinal vessels and of pulsating veins. *Roy. Lond. Ophthal. Hosp. Rep.* **12**, 59-60.
- MANN, I. (1937). *Developmental abnormalities of the eye*. Cambridge University Press.
- NETTLESHIP, E. (1905). Notes on blood vessels of the optic disc in some lower animals. *Trans. ophthal. Soc. U.K.* **25**, 338-359.
- PICKWORTH, F. A. (1934). A new method of study of brain capillaries and its application to the regional localization of mental disorder. *J. Anat., Lond.*, **69**, 62-71.
- POIRIER, P. & CHARPY, A. (1912). *Traité d'anatomie Humaine*, tome 5, fasc. II. Paris: Masson.
- SAUTER, H. & SEITZ, R. (1952). Untersuchungen über die Beziehungen zwischen Zentral- und Ciliargefäßsystem im Bereich der Lamina Cribrosa. *Graefes Arch. Ophthal.* **152**, 413-424.
- TRAQUAIR, H. M. (1946). *An introduction to clinical perimetry*. London: Kimpton.
- WOLFF, E. (1939). Some aspects of blood supply of the optic nerve. *Trans. ophthal. Soc. U.K.* **59**, 157-162.
- WOLFF, E. (1940). The blood supply to lamina cribrosa (a correction). *Trans. ophthal. Soc. U.K.* **60**, 69.
- WOLFF, E. (1954). *Anatomy of eye and orbit*, 4th ed. London: Lewis.
- XUEREB, G. P., PRITCHARD, M. L. & DANIEL, P. M. (1954). Arterial supply and venous drainage of the human hypophysis cerebri. *Quart. J. exp. Physiol.* **39**, 199-217.





STEELE AND BLUNT—THE BLOOD SUPPLY OF THE OPTIC NERVE AND CHIASMA IN MAN

EXPLANATION OF PLATES

Key to abbreviations used in text-figures and in Pls. 1 and 2

<i>a.c.</i>	anterior cerebral artery.	<i>i.c.</i>	internal carotid artery.
<i>a.co.</i>	anterior communicating artery.	<i>o.a.</i>	ophthalmic artery.
<i>a.c.z.</i>	arterial circle of Zinn.	<i>O.N.</i>	optic nerve.
<i>a.h.</i>	anterior superior hypophyseal artery.	<i>p.c.</i>	posterior ciliary artery.
<i>C.R.A.</i>	central retinal artery.	<i>p.co.</i>	posterior communicating artery.
<i>C.R.V.</i>	central retinal vein.	<i>p.h.</i>	posterior superior hypophyseal artery.
<i>d.s.</i>	dural sheath.	<i>p.p.</i>	arterial branch to pial plexus.
<i>I.</i>	infundibulum.	<i>r.b.</i>	recurrent branch of anterior superior hypophyseal artery.

PLATE 1

- Fig. 1. Lower surface of chiasma. Micropaque injection.
- Fig. 2. Upper surface of chiasma. Neoprene injection.
- Fig. 3. Longitudinal section of the anterior end of optic nerve. Sodium nitroprusside-benzidine preparation.
- Fig. 4. Vessels of the right optic nerve seen from below. In this specimen the central retinal artery is a branch of a posterior ciliary artery. Neoprene injection.
- Fig. 5. Two intraneural branches of central retinal artery from specimen in fig. 4.

PLATE 2

- Fig. 6. Horizontal section of chiasma passing through the hypothalamus. Note third ventricle (III) and periventricular capillary plexus. Sodium nitroprusside-benzidine preparation.
- Fig. 7. Longitudinal section through intraorbital part of nerve, showing box pattern of glial septa (cf. fig. 10). Van Gieson's stain.
- Fig. 8. Longitudinal section through intracranial part of nerve, showing irregular form and spiral twisting of capillaries (cf. fig. 11). Sodium nitroprusside-benzidine preparation.
- Fig. 9. Transverse section through intraorbital part of nerve. Sodium nitroprusside-benzidine preparation.
- Fig. 10. Longitudinal section through intraorbital part of nerve, showing box pattern of capillaries (cf. fig. 7). Sodium nitroprusside-benzidine preparation.
- Fig. 11. Longitudinal section through intracranial part of nerve, showing irregular form and spiral twisting of glial septa (cf. fig. 8). Van Gieson's stain.

A QUANTITATIVE STUDY OF THE POSTNATAL CHANGES IN THE PACKING DENSITY OF THE NEURONS IN THE VISUAL CORTEX OF THE MOUSE

By M. HADDARA

Department of Anatomy, University College, London

INTRODUCTION

Previous studies on the ontogenetic development of the cerebral cortex have shown that the increase in the volume of the cortex as age advances is accompanied by the spacing out of cortical neurons (Vignal, 1888; Isenschmid, 1911; Sugita, 1918*a, b*; Conel, 1939, 1941, 1947, 1951; Peters & Flexner, 1950). The purpose of this paper is to study this phenomenon quantitatively in the visual cortex of the mouse.

MATERIAL

Breeding

The animals used in this study were descendants of C57 black mice obtained from the Department of Genetics, University College, London, through the kindness of Dr H. Grüneberg. For breeding purposes several adult males and females from different litters were left together in one cage; the pregnant females were subsequently isolated.

Determination of age

The isolated females were inspected for litters in the morning and the late afternoon. The animals were considered newly born when they were first found. This implies that the real age of the animal may be up to 16 hr. more than the age attributed to it in this work.

Determination of the visual area

Several investigators have produced cytoarchitectonic maps for the mouse cortex (Isenschmid, 1911; de Vries, 1912; Fortuyn, 1914; Rose, 1929). All except Isenschmid identified a visual area on the posterior part of the lateral aspect of each hemisphere. These investigators agree with Brodmann's localization of the visual cortex in his cytoarchitectonic map for the rodents (1909). Studies on the brain of rat by Lashley (1934) and Waller (1934) support the conclusions based on cytoarchitectonic studies.

METHODS

Histological technique

The animals were anaesthetized with ether and perfused with saline followed by Bouin's fluid. Fixation was completed in Bouin. The brains were embedded in paraffin and cut at 10 and 15 μ ; the sections were then stained with gallocyenin.

Twenty-five counts on the visual cortex were made from eleven animals representing five different ages. Estimates were made of the density of neurons and of the dimensions of the cell bodies. These were used to calculate (a) the mean volume of the perikarya at different ages, and (b) the actual number of neurons in a known volume of tissue. These numbers were determined from the crude counts by the method developed by Abercrombie (1946).

Counting

The numbers of perikarya and perikaryal fragments of the neurons in rectangular strips of cortex between the pia and the white matter were counted under a binocular microscope, using a $\times 40$ objective and $\times 10$ eyepieces. The counts of the 3-day-old mouse were made with an oil-immersion objective. Care was taken to ensure that the long axis of each strip was parallel to the pial surface. The counts were made by means of a grid in one of the eyepieces of the microscope.

Since the molecular layer contains very few nerve cells, this study has concentrated on the submolecular region of the cortex. It is necessary therefore to define clearly the boundary lines limiting the submolecular cortex on the counts.

Boundaries of the submolecular cortex

When the outermost line of the ocular grid was orientated along the pial surface, the lowest row of squares of the grid covering the cortex was often only partially occupied by cells; consequently, some approximation was necessary in order to define the lower boundary of the cortex. In the 3-day-old animal the approximation was made to one row, i.e. if the cells occupied half or more than half of the row, this was considered as a complete row, and if, on the other hand, the cells occupied less than half of the row the number of cells was added to the total found in the previous row. In animals 7 days old or older the approximation was to half a row, since the thickness of the row in these cases was nearly twice that found in the 3-day-old stage.

In cases where the molecular layer occupied half or more than half the thickness of a row that was partly in the submolecular cortex, this row was considered to lie wholly in the molecular layer. But if the molecular layer occupied less than half the thickness of that row, it was considered to fall wholly in the submolecular cortex.

Measuring the dimensions of the cell bodies

Two measurements were taken for each perikaryon: the maximum length (l) along the long axis, and the maximum breadth (d) perpendicular to the long axis. For each age studied at least eighty cells were measured. The thickness of the cells, i.e. the dimension perpendicular to the plane of the section, was considered to be equal to the breadth (d) of the cell.

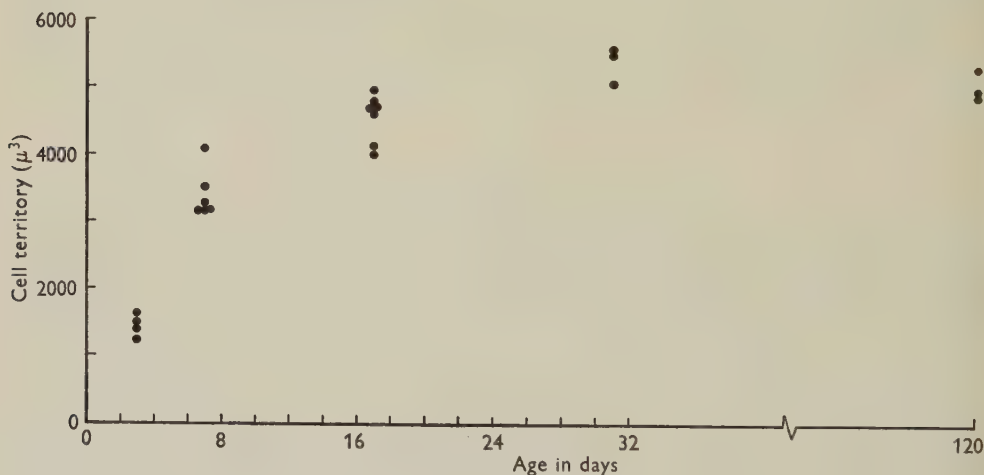
The mean measurements, \bar{l} and \bar{d} , were calculated. These must be less than the true mean cellular dimensions, because one cannot differentiate whole perikarya from fragments. However, this error is not great, and was estimated by Abercrombie (1946) as being of the order of 6%.

These means were used to estimate:

(a) The mean volume of perikarya at various ages, by treating the perikaryon as a spheroid, using the formula $\frac{4}{3}\pi ab^2$, where $a = \frac{1}{2}\bar{l}$ and $b = \frac{1}{2}\bar{d}$.

(b) The actual number of cells in a particular cortical strip, correcting by Abercrombie's method.

The series of photographs in Plate 1 show five developmental stages of the visual cortex of the mouse in postnatal life, stained with gallocyanin. Examination of this series shows that with increasing age the cortex becomes broader and the perikarya of the neurons increase in size, becoming less densely packed. Three cortical zones (apart from the molecular layer) can be distinguished in all stages: a superficial zone of densely packed cells, a sparsely populated middle zone, a deep zone of more densely packed cells. The contrast in density between the superficial and the deep zones decreases gradually with age.



Text-fig. 1. The changes in the volume of cell territory with increasing age.

Changes in the packing density of cortical neurons during postnatal development

A. Increase of cell territory with age

The cell territory (S) for a particular cortical 'strip' is defined as the ratio of the total cortical volume in μ^3 to the number of whole neurons it contains. The greater the spacing between the cortical neurons the greater would be the cell territory in that strip.

B. The growth of the cell territory

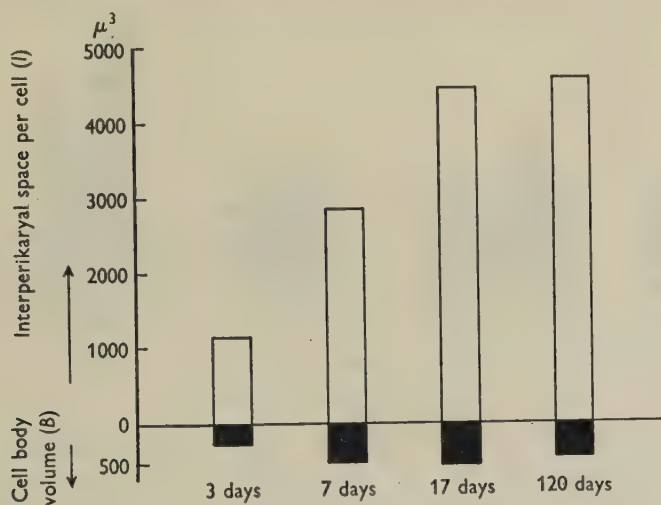
The cell territories (S) for twenty-five counts for four developmental stages in postnatal life were plotted against age (Text-fig. 1). The curve rises steeply between the age of 2 and 7 days, then becomes less steep between 7 and 17 days. After this stage it continues almost parallel with the time axis. This means that sizes of the spaces between the nerve cells of the cortex increase rapidly from 3 to 7 days, more slowly between 7 and 17 days, and almost cease to change after that time.

Table 1. *Components of the cell territory of the neurons in the cerebral cortex of the mouse*

Age (days)	Mean volume of the perikarya (μ^3)	Mean cell territory (μ^3)	Interperikaryal space per cell (μ^3)
3	281	1497	1216
7	535	3375	2840
17	576	5058	4482
120	467	5068	4601

C. Components of the cell territory

The cell territory as defined above contains the perikaryon together with a volume of interperikaryal space, the cortical tissue between the perikarya of the nerve cells as seen in preparations stained by gallocyanin. Table 1 gives the mean volumes of the perikarya and the mean cell territories found at different ages. In Text-fig. 2 the



Text-fig. 2. The changes in the components of the cell territory with increasing age.

length of each column represents the mean cell territory at a particular stage of development (based on all the counts for that age). The length of the black part of the column is proportional to the mean volume of the perikarya, while the length of the unshaded part of the column is proportional to the mean volume of the interperikaryal space per cell, at that particular stage of development. It is clear that the increase in cell territory that occurs in the growing cortex is mainly the result of the increase in interperikaryal space.

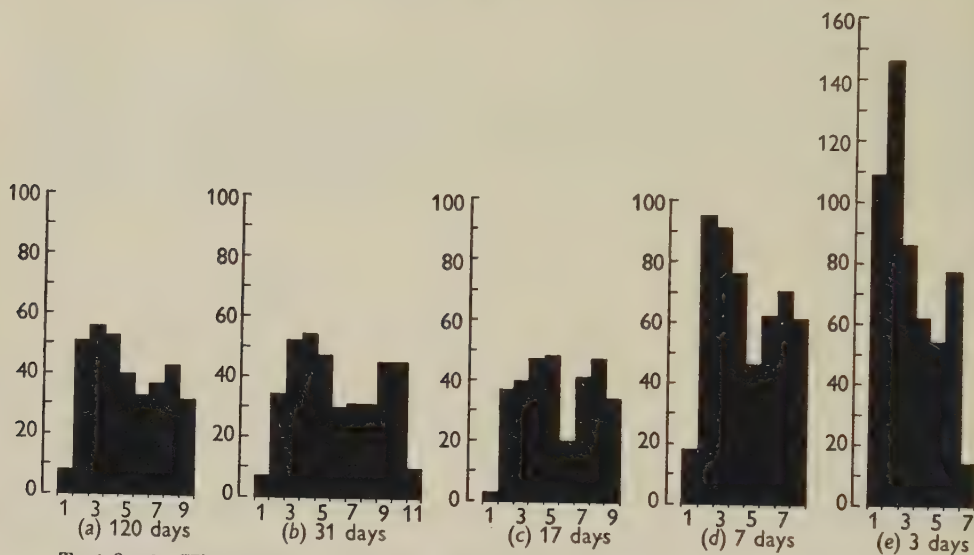
In the adult the interperikaryal space per cell forms about 90% of the cell territory; in other words, the cortical tissue between the perikarya forms 90% of the volume of cortex considered.

D. Variation in the packing density with depth

The photographs in Pl. 1 show that the packing density of the cells is not the same at all depths of the cortex at any of the ages studied. The series of histograms

in Text-fig. 3 illustrates the change in the number of neurons with depth in the visual cortex of mice in five developmental stages. Histograms *a*, *b*, *c* and *d* each represent a strip of cortex 150μ wide, and histogram *e*, which belongs to a 3-day-old stage, represents a cortical strip 140μ wide.

The number of neurons counted is shown vertically, and each horizontal unit is equivalent to an interval of 75μ in histograms *a*, *b*, *c* and *d*, and to an interval of 70μ in histogram *e*. The pial surface was taken as the origin.



Text-fig. 3. Histograms showing the distribution of the number of neurons in relation to their depth within the cortex.

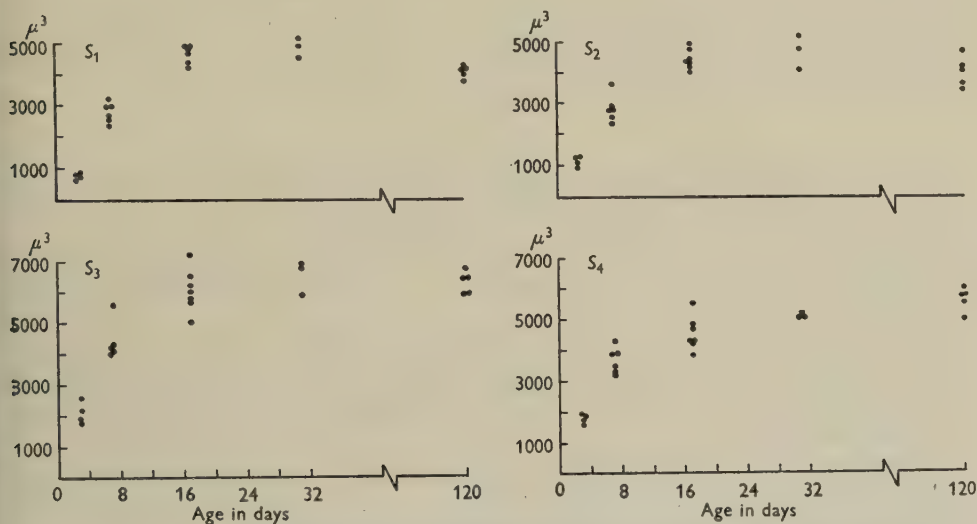
All five histograms show two peaks, separated by a trough. The peaks are high in the 3-day stage, but gradually become lower as age advances, and after the 17-day stage there is little change in their height. The difference between the first and the second peaks is greatest in the 3-day mice, but this difference in height decreases with age.

Briefly, the spaces between the neurons of the cortex increase with the age, the amount of the increase being related to the depth in the cortex at which the neurons are situated. This variation may be examined more precisely by comparing the cell territories at corresponding depths throughout the whole series.

The submolecular region of each cortical strip was divided into four zones for this purpose in such a manner that each zone contained 25% of the total number of cells present in the cortical strip. The cell territories in each of the four zones of each strip were calculated. These were termed S_1 , S_2 , S_3 and S_4 , from the surface inwards.

Text-fig. 4 shows the result of plotting S_1 , S_2 , S_3 and S_4 against age. The patterns of the plotted points closely resemble those describing the average increase in cell territory throughout the total depth (Text-fig. 1). It may be concluded that in all four zones of the cortex there is a marked increase in the cell territory in the first 2 weeks of postnatal life. This increase is greatest in the first week and almost ceases after the seventeenth day.

The increase in the cell territory from 3 to 120 days is almost the same in the four zones and ranges from 4000 to $5000\mu^3$, but the extent of the cell territory at the earliest age studied varied between the different zones, being $800\mu^3$ in the outermost zone and $2000\mu^3$ in the third zone. The rates of increase in cell territory are consequently higher in the outer zones. The deeper zones show a greater volume of cell territory earlier in the life of the animal; this may indicate an earlier maturation in the deeper parts of the cortex.



Text-fig. 4. The changes in the magnitude of the cell territory at different depths of the cortex with increasing age.

DISCUSSION

The changes that take place in the organization of the cerebral cortex during development have been studied by many workers. The histological changes have been studied by Bolton (1903), Cajal (1911) and Lorente de Nó (1933), to name only a few investigations. Other workers have studied changes in the electrical activity of the growing cortex, for example, Lindsley (1936), Bishop (1950) and Hunt & Goldring (1951). The recent studies from Prof. Flexner's laboratory (Peters & Flexner, 1950; Flexner & Flexner, 1948; Flexner, Tyler & Gallant, 1950) have investigated the correlated histological, electrical and biochemical changes in the growing cortex of the guinea-pig.

The present work is a quantitative study of the changes in the packing density of the perikarya of the neurons in the developing visual cortex of mice. With each neuron is associated a 'cell territory', that is, an average measure of the volume of cortex apportioned to the perikarya of each neuron. It comprises the volume of the perikaryon itself, together with 'interperikaryal space'.

The packing density of the neurons decreases rapidly in the first week after birth, and then slows down until no change is found after the seventeenth day. The corresponding increase in cell territory is mainly due to an increase in the interperikaryal space. This increase in interperikaryal space presumably results from the growth of

the axons and dendrites of the cortical neurons and the ramifications of axons from the white matter (Stefanowska, 1898; Cajal, 1911; Lorente de N6, 1933).

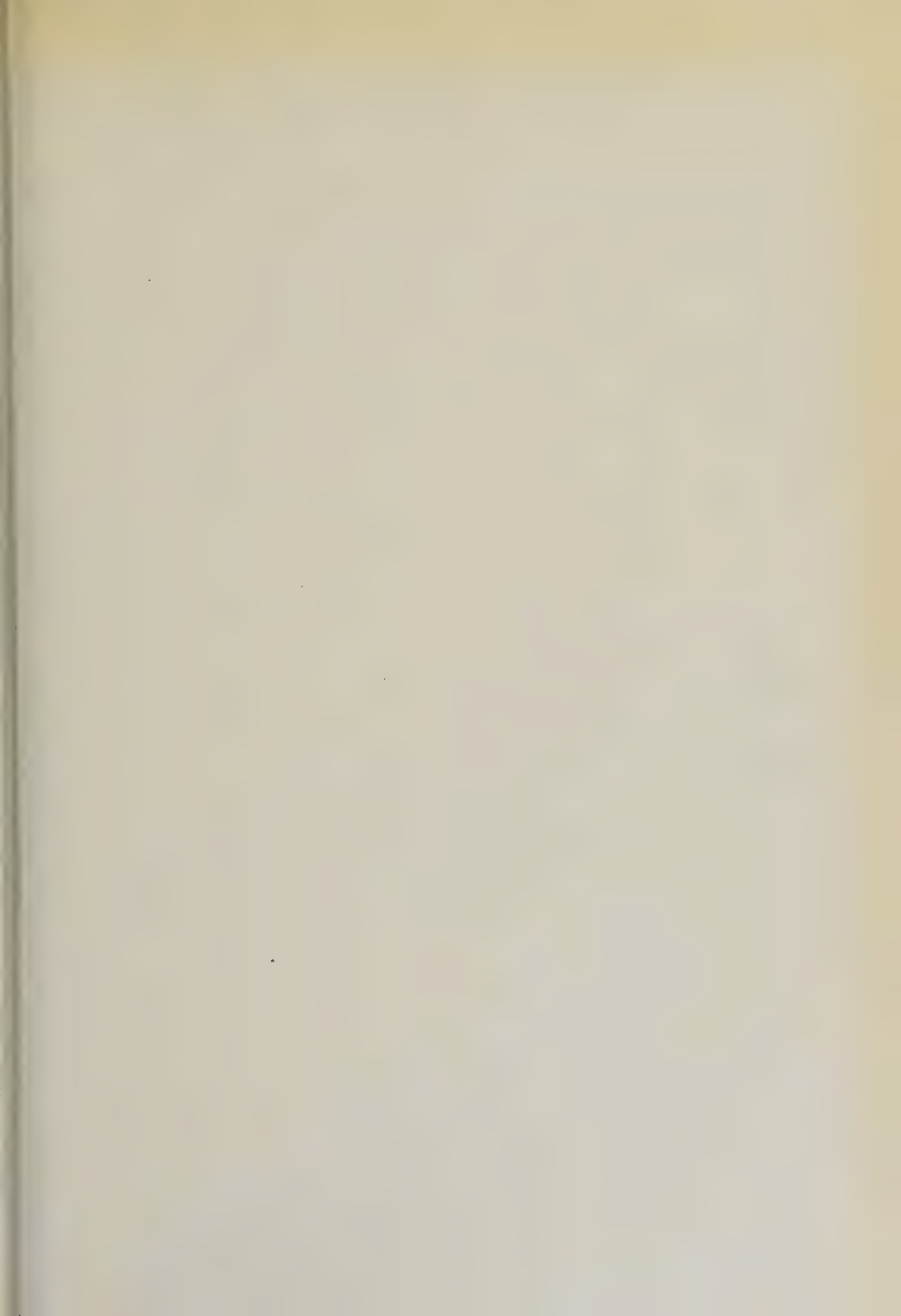
Bolton (1903) and Watson (1907), who studied the development of the cortex in man and small mammals qualitatively, divided the cortex into supragranular, granular and infragranular layers. They concluded that the infragranular layer became mature before the supragranular layer. A similar result was found in foetal pigs by Flexner, Flexner & Straus (1940), who noted that increased spacing of the neurons first appears in the deeper part of the cortex. The present work not only quantifies these results but shows that the magnitude of this increase at different depths of the cortex is independent of depth, although the increase in cell territory takes place earlier in the deeper parts of the cortex.

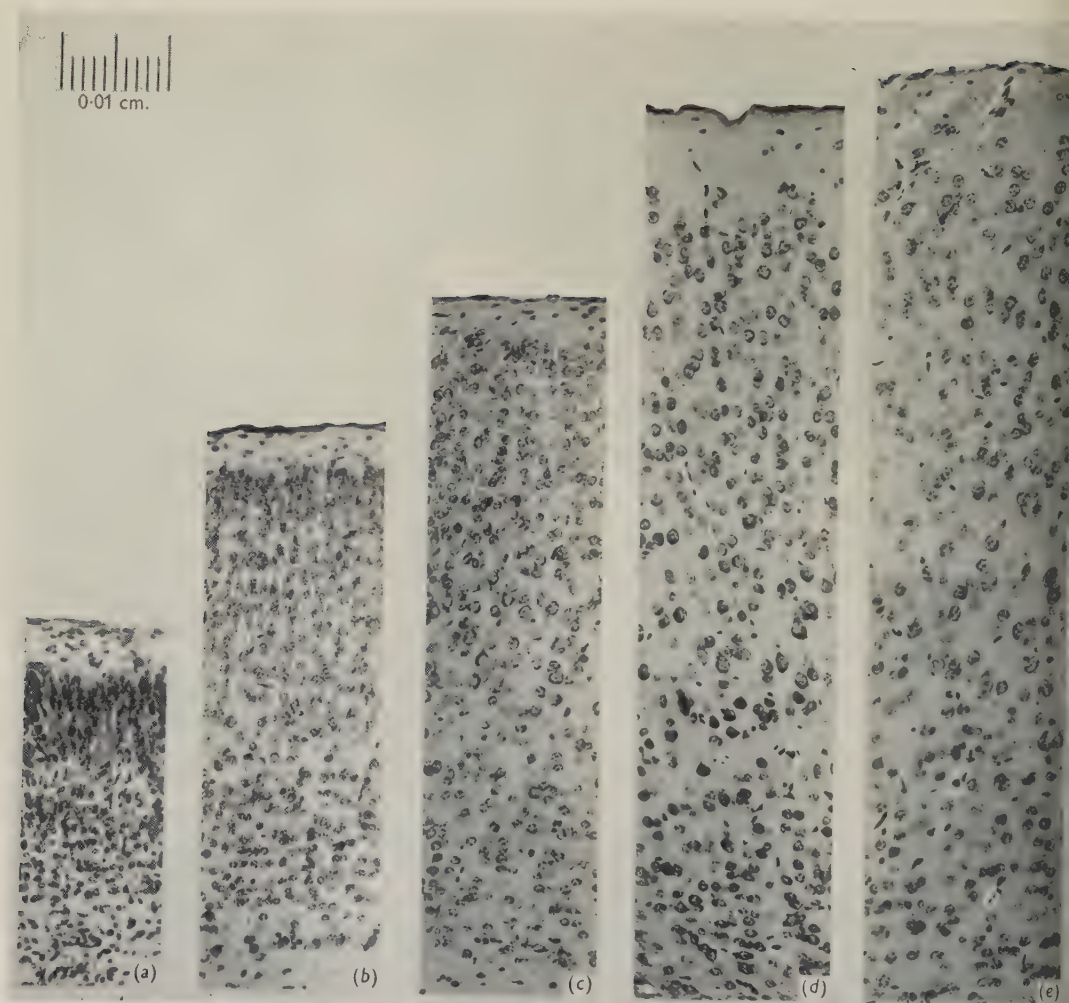
SUMMARY

1. The postnatal changes in the neuronal organization of the visual cortex of the mouse were studied in preparations stained with gallocyanin.
2. The packing density of the neurons decreases rapidly between the third and seventh days after birth and then more slowly, no change taking place after the seventeenth day.
3. The cell territory of a neuron was defined as the ratio between the volume of cortex considered and the number of neurons contained in that volume. The cell territory defined in this way comprises the volume occupied by the perikaryon and the interperikaryal space.
4. The greater part of the decrease in packing density of the neurons is due to the increase of the interperikaryal space. The increase in the size of the perikarya is only responsible for a small part of the change in cell territory.
5. The total increase in cell territory is the same at all depths of the cortex, but this increase occurs earlier in the deeper parts of the cortex.

REFERENCES

- ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239-247.
- BISHOP, E. J. (1950). The strychnine spike as a physiological indicator of cortical maturity in the postnatal rabbit. *Electroenceph. clin. Neurophysiol.* **2**, 309-315.
- BOLTON, J. S. (1903). The histological basis of amentia and dementia. *Arch. Neurol., Lond.*, **2**, 424-620.
- BRODMANN, K. (1909). *Vergleichende Lokalisationslehre der Grosshirnrinde*. Leipzig.
- CAJAL, S. R. (1911). *Histologie du système nerveux de l'homme et des vertébrés*, vol. 2. Paris.
- CONEL, J. LE ROY (1939-51). *The postnatal development of the human cerebral cortex*, vols. 1-5. Cambridge, Massachusetts.
- FLEXNER, J. B. & FLEXNER, L. B. (1948). Biochemical and physiological differentiation during morphogenesis. VII. Adenylpyrophosphatase and acid phosphatase activities in the developing cerebral cortex and liver of the fetal guinea pig. *J. cell comp. Physiol.* **31**, 311-320.
- FLEXNER, J. B., FLEXNER, L. B. & STRAUS, W. L. (1941). The oxygen consumption, cytochrome and cytochrome oxidase activity and histological structure of the developing cerebral cortex of the fetal pig. *J. cell. comp. Physiol.* **18**, 355-368.
- FLEXNER, L. B., TYLER, D. B. & GALLANT, L. J. (1950). Biochemical and physiological differentiation during morphogenesis. X. Onset of electrical activity in developing cerebral cortex of fetal guinea-pig. *J. Neurophysiol.* **13**, 427-430.
- FORTUYN, A. B. D. (1914). Cortical cell-lamination of the hemispheres of some rodents. *Arch. Neurol. Psychiat., Lond.*, **6**, 221-354.





HADDARA—POSTNATAL CHANGES IN THE VISUAL CORTEX OF THE MOUSE

(Facing p. 501)

- HUNT, W. E. & GOLDRING, S. (1951). Maturation of evoked response of the visual cortex in the postnatal rabbit. *Electroenceph. clin. Neurophysiol.* **3**, 465-471.
- ISENSCHMID, R. (1911). Zur Kenntnis der Grosshirnrinde der Maus. *Abh. preuss. Akad. Wiss.* **8**, 1911, pp. 1-78.
- LASHLEY, K. S. (1934). The mechanism of vision. VIII. The projection of the retina upon the cerebral cortex of the rat. *J. comp. Neurol.* **60**, 57-79.
- LINDSLEY, D. B. (1936). Brain potentials in children and adults. *Science*, **84**, 354.
- LORENTE DE NÓ, R. (1933). Studies of the structure of the cerebral cortex. *J. Psychol. Neurol., Lpz.*, **45**, 381-438.
- PETERS, V. B. & FLEXNER, L. B. (1950). Biochemical and physiological differentiation during morphogenesis. VIII. Quantitative morphologic studies of the developing cerebral cortex of the fetal guinea-pig. *Amer. J. Anat.* **86**, 133-161.
- ROSE, M. (1929). Cytoarchitektonischer Atlas der Grosshirnrinde der Maus. *J. Psychol. Neurol., Lpz.*, **40**, 1-51.
- STEFANOWSKA, M. (1898). Evolution des cellules nerveuses corticales chez la souris après la naissance. *Trav. Lab. Physiol. Inst. Solvay* 1898, T.2-F.2, pp. 1-44.
- SUGITA, N. (1918*a*). Comparative studies on the growth of the cerebral cortex. V. *J. comp. Neurol.* **29**, 61-117.
- SUGITA, N. (1918*b*). Comparative studies on the growth of the cerebral cortex. VII. *J. comp. Neurol.* **29**, 177-240.
- VIGNAL, W. (1888). Recherches sur la développement de la substance corticale du cerveau et du cervelet. *Arch. Physiol. norm. path.* **2**, 311-338.
- DE VRIES, I. (1912). Über die Zytoarchitektonik der Grosshirnrinde der Maus und über die Beziehungen der einzelnen Zellschichten zum Corpus Callosum auf Grund von experimentellen Läsionen. *Folia neuro-biol., Lpz.*, **6**, 289-322.
- WALLER, W. H. (1934). Topographical relations of cortical lesions to thalamic nuclei in the albino rat. *J. comp. Neurol.* **60**, 237-269.
- WATSON, G. A. (1907). The mammalian cerebral cortex, with special reference to its comparative histology. 1. Order Insectivora. *Arch. Neurol., Lond.*, **3**, 49-122.

EXPLANATION OF PLATE

Sections of the visual cortex of the mouse at different ages. Galloeyanin stain. (a) Newly born; (b) 3 days old; (c) 7 days old; (d) 17 days old; (e) 31 days old.

THE ULTRASTRUCTURE OF THE SYNAPTIC AREA IN THE SUPERIOR CERVICAL GANGLION

BY G. CAUSEY AND H. HOFFMAN

Department of Anatomy, Royal College of Surgeons of England

Electron microscopic studies of nerve-cells and nerve-fibres (Causey & Hoffman, 1955, 1956) have emphasized the exceedingly intimate relationship between neurone and Schwann or satellite cell along their entire length. These studies have been extended to the relationships between neurones, axons and adventitious cells in the superior cervical ganglion in normal material and after section of the preganglionic fibres.

The mode of connexion between neurones has long been debated. Although the work of Cajal (1893), summarized in his definitive work (1909) and reviewed by Nonidez (1944), established to the satisfaction of most workers that the connexion is by contact rather than continuity, there have always been some workers who assert that some form of protoplasmic continuity exists across the synapse (Boeke, 1940; Stöhr, 1932; Tiegs, 1926; Hillarp, 1946). Even if we accept the Cajal interpretation, another point arises in the work of Couteaux (1945) and de Castro (1951). These investigators have claimed that an adventitious element (Schwann, glial or satellite) intervenes between the neuronal components of the synapse. Couteaux, in particular, has postulated that an adventitious element, the 'teloglia', is present between the active conducting elements, both in central synapses and at the motor end-plate.

In the last few years several electron microscopic studies have been made on the synaptic region in various sites. As regards the internal composition of the component neural processes, some agreement has been reached: de Robertis & Bennett (1954) describe a concentration of mitochondria and small vesicles in the presynaptic axon, and Palade (1954) describes similar structures in the axodendritic synapses of the cerebellar cortex. Close approximation of the synaptic elements is indicated by Palade (1954), Wyckoff & Young (1954), Sjöstrand (1954) and Estable, Reissig & de Robertis (1954). The precise relationship of the membranes has been indicated only in certain types of synapses; the axodendritic endings of the cerebellar cortex (Palade, 1954) and endings on retinal rods (Sjöstrand, 1954), and the median to motor synapses of the crayfish (Robertson 1954, 1955) in which the double-layered membranes of the two contiguous cells are within 200 A.U. of each other, but not fused.

The superior cervical ganglion was chosen for the study of the relation on these structures in view of our previous study of the dorsal root ganglion (Causey & Hoffman, 1955) and because the clear-cut relation between the collagen-filled tissue space, the satellite cell capsule and the ganglion cell makes it easier to interpret the cellular relationships than in the central nervous system.

MATERIAL AND METHODS

Superior cervical ganglia of three normal rabbits were removed under urethane anaesthesia, placed on a chilled glass plate and cut into very small pieces which were then fixed in 1% osmium tetroxide buffered to approximately 7.2–7.4 pH, for 4 hr. at about 4° C. They were embedded in butyl methacrylate polymerized at room temperature by ultraviolet light, and sectioned at 250–500 A.U. thickness on a Cook and Perkins thermal expansion microtome.

In two rabbits the preganglionic sympathetic chain on one side was sectioned under Nembutal anaesthesia (35 mg./kg.). Operated and control ganglia were removed under anaesthesia and fixed as described above, 8 and 16 days after operation.

Sections were examined and photographed in a Metropolitan-Vickers EM4 electron microscope, equipped with a 50 μ objective aperture, at primary magnifications of $\times 4000$ –5000.

OBSERVATIONS

The cytoplasm of the neurones of the superior cervical ganglion (Pl. 1, fig. 1) consists essentially of rather small Nissl aggregates of approximately 100 A.U. granules, and mitochondria of various sizes, from 0.2 to 1.5 μ in greatest dimension, the larger ones being well illustrated in Pl. 4, fig. 8. Simple vesicles also occur, and some with very short crista-like invaginations are seen in Pl. 1, fig. 2, alongside typical mitochondria, and in these respects closely resemble the cytoplasm of sensory ganglion cells (Causey & Hoffman, 1955). Clumps of densely stained liposomes are seen usually in juxtanuclear position, but occasionally reaching the cell surface as in Pl. 2, fig. 3.

The satellite cells also resemble those described previously in the sensory ganglia. Their free edge is marked by a membrane as is their surface of contact with the ganglion cell; this is shown in Pl. 1, figs. 1, 2 and Pl. 2, figs. 3, 4. The entire ganglion cell is invested in this fashion by a capsule formed of these cells; where two satellite cells meet there are two sets of double membranes, separated by a space of a few hundred A.U. The cytoplasm of the satellite cells contains numerous, often quite large mitochondria, and clumps of small vesicles in the 200–500 A.U. range. At various places in the satellite cell, sometimes near the nucleus, sometimes well separated from it, the cytoplasm increases to form a hillock, within which numerous presynaptic axons may be seen; such an area is seen in most of the figures, it is particularly well shown in Pl. 3, figs. 5, 6. As may be seen in these illustrations, the axons are surrounded by double membranes, with spacing between 120 and 160 A.U., but they vary somewhat in internal composition. Sometimes, as in Pl. 2, fig. 4, Pl. 3, figs. 5, 6 and Pl. 4, fig. 8, they resemble the non-myelinated axon seen more proximally, with a few mitochondria, and typical protofibrils shown in oblique section in Pl. 4, fig. 8. Sometimes, however, they are packed with mitochondria, as in Pl. 1, figs. 1, 2 and Pl. 4, fig. 7, and small vesicles, ranging in size from 200 to 500 A.U., shown in Pl. 1, figs. 1, 2, Pl. 2, fig. 4 and Pl. 4, fig. 7. The presynaptic axons vary in size from 0.2 to 2 μ and are of great interest in their passage within the satellite cells. Their distance from the ganglion cell surface is variable, but at this

stage they are all contained within the cytoplasm of what may be considered as their last Schwann or adventitious cell.

The double membrane around the axon sometimes lies within 200 A.U. of the membrane between satellite and ganglion cell, as described by Palade. This may be seen in Pl. 1, figs. 1 and 2, and Pl. 4 fig. 8. Frequently the space is much greater, as may be seen in cases of other axons in the same figures. In such cases all the component elements of satellite cytoplasm lie between the presynaptic elements, and often numerous layers of double membrane lie within this space. These membranes are of similar spacing to those of the satellite cell surfaces, sometimes occurring as parallel straight lines, but often wrapping around the axon. In Pl. 1, fig. 1, Pl. 2, fig. 4 and Pl. 4, fig. 7, such parallel-arranged intervening membranes may be seen, while in Pl. 3, figs. 5, 6, the presynaptic axons are more or less surrounded by multiple membranes. Some of these membranes would appear to be comparable with the mesaxons described by Gasser (1952, 1955) and ourselves (Causey & Hoffman, 1956) which connect the membranes of the axon and Schwann cell. They are, in fact, invaginations of satellite cell membrane—coming either from the free surface or surface of contact with the ganglion cell. Sometimes they form small or large coils, in association with a presynaptic axon, as in Pl. 2, fig. 4, but the most striking formation is the concentric whorl or spiral. Such a structure is shown in Pl. 4, fig. 9, but may also be identified in Pl. 1, figs. 1, 2 and Pl. 2, fig. 3, while the connexion of such a structure with the surface membrane is shown in Pl. 4, fig. 10, where an invagination from the inner satellite-ganglion cell membrane is seen to form a spiral. Such concentric spirals are described by Robertson (1955) in the Schwann-synaptic region in the crayfish.

The concentric wrapping of the presynaptic axon by these membranes, the occurrence of invaginating spirals and the parallel sheets seen in some cases may presumably be due to different planes of section of a cylindrical sheet forming a series of concentric laminae round the axon, fusing in places, and extending beyond the axon termination. In support of the suggestion that the invaginations are sheets at least in some cases, serial sections from a synaptic region of a satellite cell are shown, in Pl. 3, figs. 5, 6. Several sections intervene between the two shown, and it will be noted that the presynaptic axons differ slightly in position and mitochondrial shape—nevertheless, the same membranes can be identified in both. The possibility of tubular forms existing is supported by the appearance of oval and circular cross-sections at some points.

In the immediate vicinity of the post-synaptic membrane no specialization of ganglion cell cytoplasm has been detected. All the component elements appeared to be present in their normal relationship, in contrast to the tendency for aggregation of mitochondria and small vesicles in the presynaptic axons.

Eight days after section of the preganglionic chain in the neck longitudinal section of the peripheral part of the chain showed the endoneurial tubes to be almost completely filled with Schwann protoplasm and within the satellite cell capsule the axons had disappeared.

At 16 days after section (Pl. 4, fig. 11) there were long stretches of satellite cell protoplasm without any axons and Schwann cells rounding off. In the central cell of Pl. 4, fig. 11, an invagination is seen that may be a mesaxon.

DISCUSSION

In this examination of the synaptic region of the superior cervical ganglion, one of the first difficulties that becomes apparent is to decide exactly when a pre-terminal axon becomes synaptic. It would appear that the situation described by some authors, of extremely close approximation of axonal and dendritic or somatic double membranes, is present in the synaptic area of superior cervical ganglion cells. However, it is obviously not the only relationship of this terminal portion of the axon, as there is often a separation of 1μ or so, added to by the wrapping round of the axon with sheets of double membrane invaginated from the free or ganglionic surface of the satellite cell.

It may be that where a gap is seen between axon and somatic surface we are in fact looking at a presynaptic portion of the axon; however, it should be pointed out that the entire portion of the axon seen within the satellite cell is close enough to the neural surface to be considered in contact according to optical microscopy, and that some of the axon endings seen 0.5μ away from the surface are more specialized internally in the mode defined by other authors as characteristic of terminals than are others within 200 A.U. of the neural surface. Finally, the axon terminals which lie in close contact with the neural surface are not wrapped in concentric membranes, while those further from it are consistently enwrapped. Whether the separation is large or small, it is clear that the environment of the presynaptic axon is that of the satellite cell cytoplasm, thus, as emphasized by Bodian (1942) the glial—or adventitious—elements may play a more active part in the function of the synaptic region than had hitherto been assumed.

Recent studies of the relationship between Schwann cell and nerve-fibre (Gasser, 1955; Causey & Hoffman, 1956) seem to indicate that the double membrane around the axon is, at least partly, of Schwann origin, and it would seem likely that the relationship between ganglion cell and satellite cell is similar. Thus it seems that the membranes which previous workers considered to form the contiguous elements of efferent and afferent sides of synapse, may be of Schwann-satellite-glial origin. The tendency for invaginations of the satellite-ganglion cell membrane to fuse with the membrane of the presynaptic axon further complicates the simple interpretation advanced by previous workers. This fusion is closely comparable with that described by Robertson (1955), and also with that encountered in nerve trunks, where the mesaxon fuses with the axon-Schwann membrane. The enclosing of the presynaptic axon in sheets of invaginated satellite membrane closely resembles the process whereby embryonic axons become myelinated by the winding around of the mesaxon (Geren, 1954).

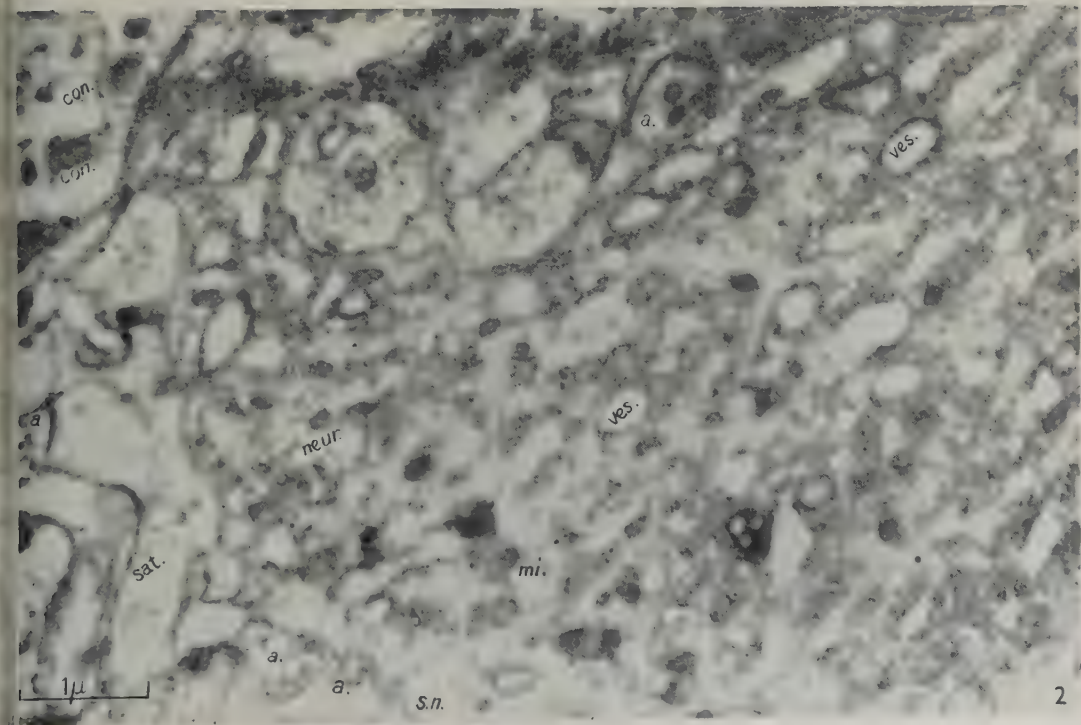
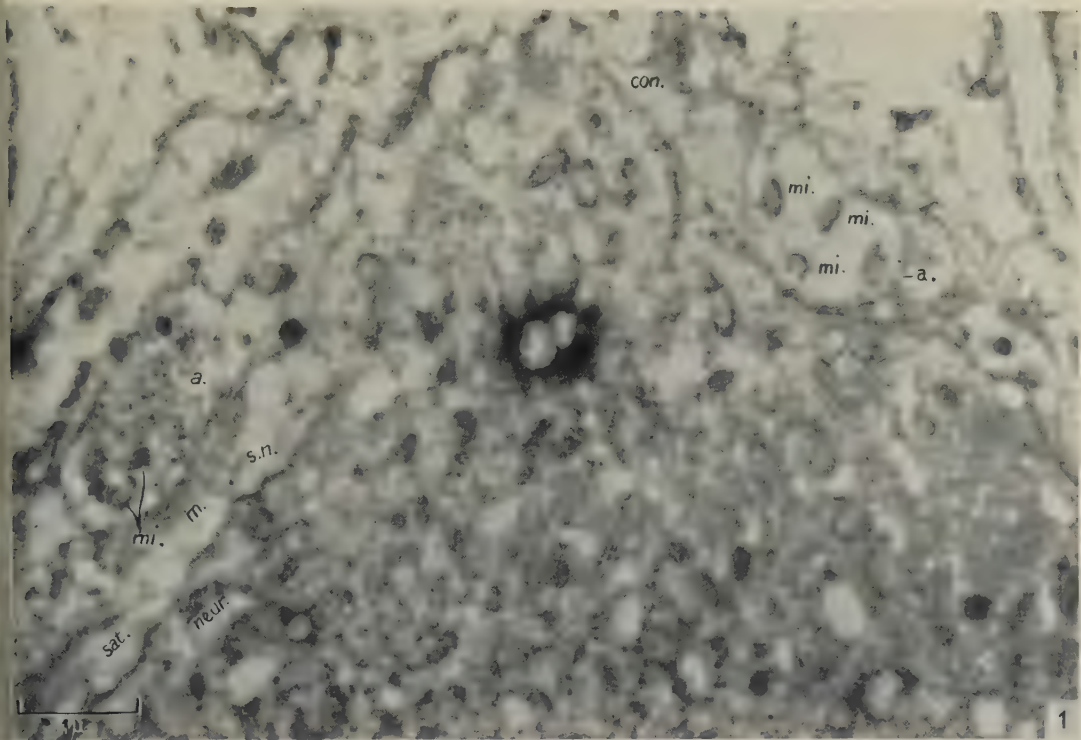
The evidence of the sections taken after preganglionic nerve section is of interest at this stage mainly as a confirmation of the validity of the interpretation of the structures seen in normal material as preganglionic fibres. Further work is needed to elucidate the cycle of change in the mesaxon. The similarity between Schwann cell and satellite cell, and their similar formation of intracellular membranes which tend to wrap around the axon, lead to the suggestion that they are, in fact, modulations of a common cell type—a cell which intimately envelops the entire neurone from cell body to termination. This would, in turn, suggest a greater participation in the physiological processes of nervous transmission.

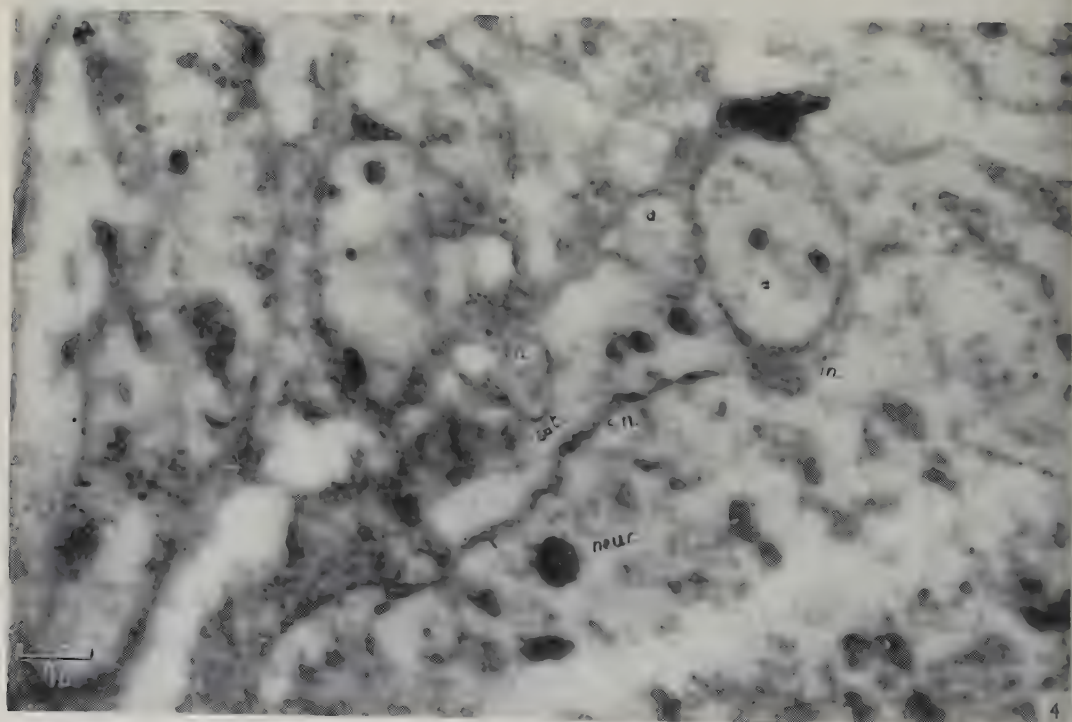
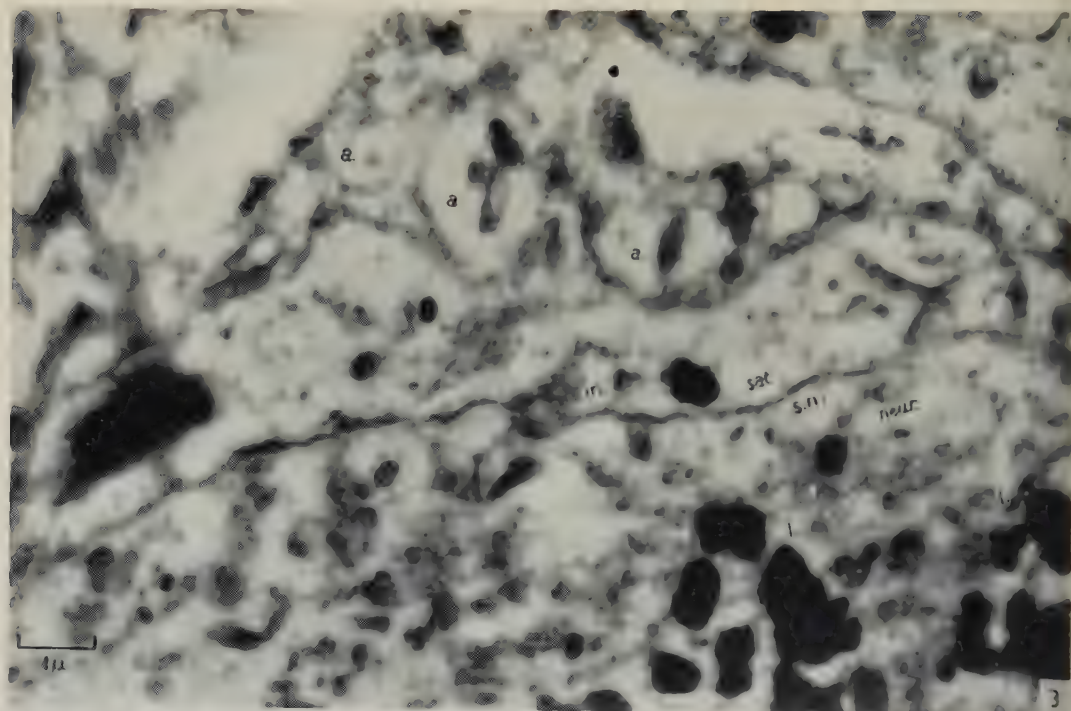
SUMMARY

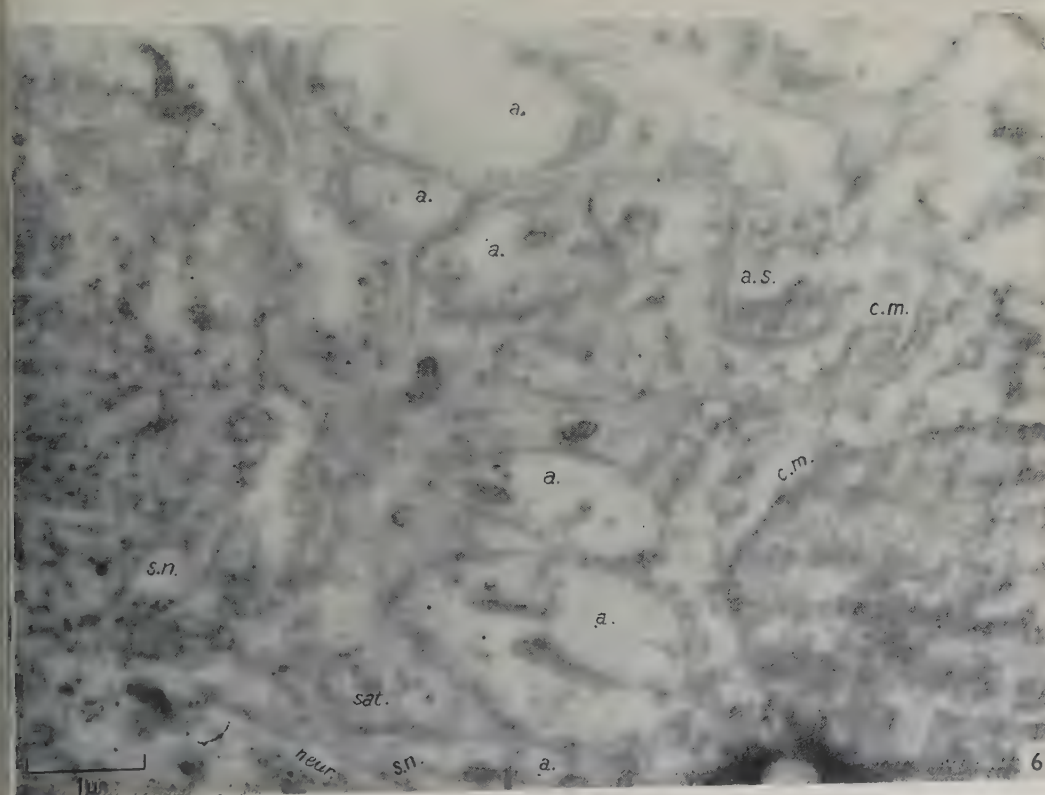
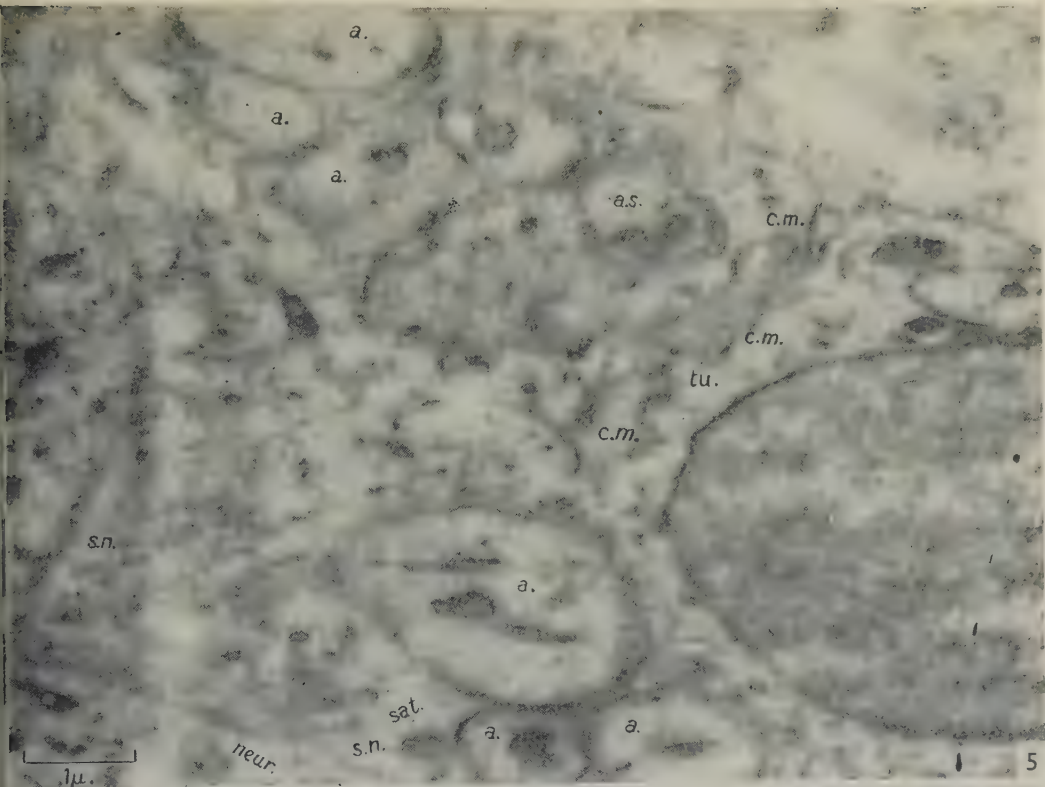
1. An examination of the superior cervical ganglion of the rabbit by electron microscopy has been made. Normal ganglia and ganglia 8 and 16 days after preganglionic section were used.
2. The terminal portion of the preganglionic fibres bears similar relations to the satellite cells of the neurones as do the fibres in a nerve trunk to the Schwann cells.
3. The variability of mitochondrial content of the terminal portions of the axons is shown and the mesaxons and concentric whorls are discussed.
4. At their site of closest apposition, the axonal and neuronal membranes are separated by an interval of some 200 Å.U.
5. After preganglionic section the axonal terminals degenerate.

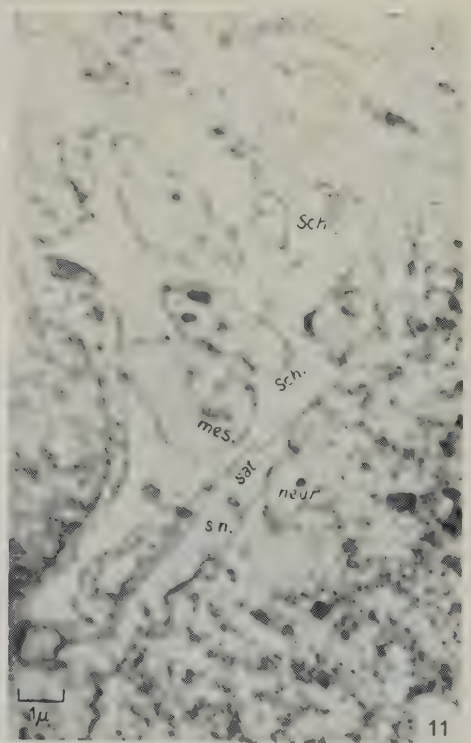
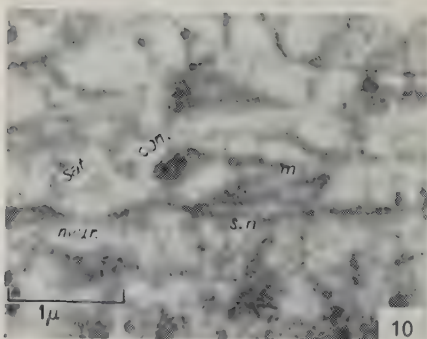
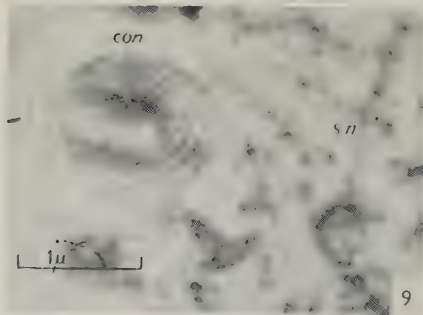
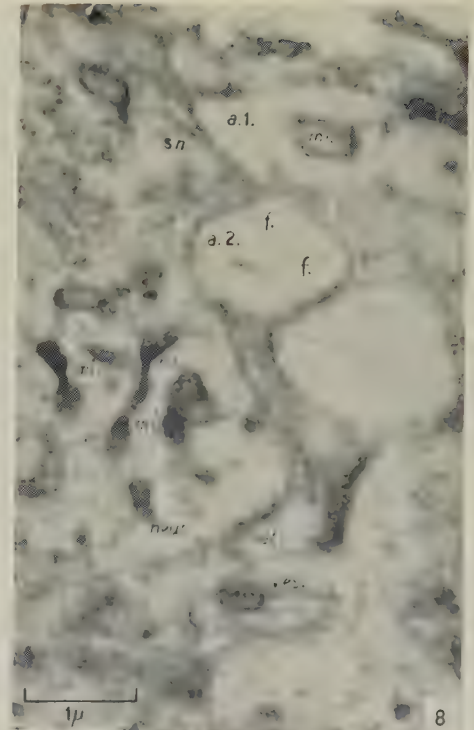
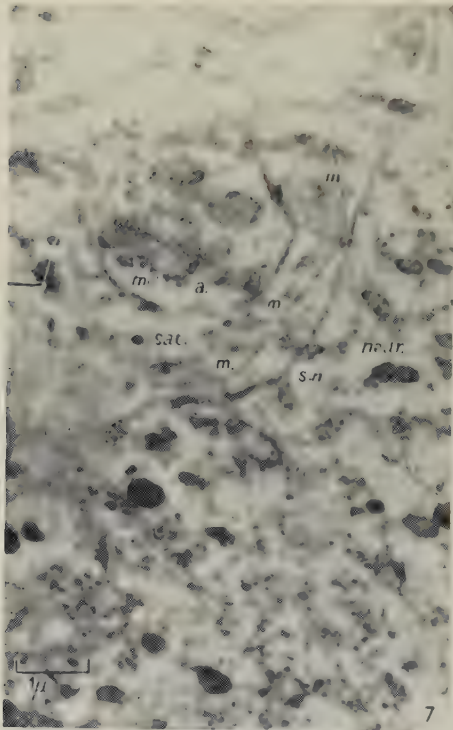
REFERENCES

- BODIAN, D. (1942). Cytological aspects of synaptic function. *Physiol. Rev.* **22**, 146-169.
- BOEKE, J. (1940). *Problems of Nervous Anatomy*. London: Oxford University Press.
- CAJAL, S. RAMON Y (1893). Neue Darstellung vom histologischen Bau des Centralnervensystems. *Arch. Anat. Physiol., Lpz. (Anat. Abt.)*, 319-428.
- CAJAL, S. RAMON Y. (1909). *Histologie du système nerveux de l'homme et des vertébrés*. Paris: Maloine.
- CAUSEY, G. & HOFFMAN, H. (1955). Cytoplasmic synthesis in nerve cells. *Brit. J. Cancer*, **9**, 666-673.
- CAUSEY, G. & HOFFMAN, H. (1956). The relation between the Schwann cell and the axon in peripheral nerves. *J. Anat., Lond.*, **90**, 1-4.
- COUTEAUX, R. (1945). La Neuroglie Terminale au niveau de la Synapse myo-neurale. *C.R. Soc. Biol., Paris*, **139**, 641-643.
- DE CASTRO, F. (1951). Aspects anatomiques de la transmission synaptique ganglionnaire chez les mammifères. *Arch. int. Physiol.* **59**, 479-525.
- DE ROBERTIS, E. & BENNETT, H. S. (1954). Submicroscopic vesicular component in the synapse. *Fed. Proc.* **13**, 35.
- ESTABLE, C., REISSIG, M. & DE ROBERTIS, E. (1954). Microscopic and submicroscopic structure of the synapsis in the ventral ganglion of the acoustic nerve. *Exp. Cell. Res.* **6**, 255-262.
- GASSER, H. S. (1952). *Cold Spr. Harb. Symp. quant. Biol.* **17**, 32-36.
- GASSER, H. S. (1955). Properties of dorsal root unmyelinated fibres on the two sides of the ganglion. *J. gen. Physiol.* **38**, 709-728.
- GEREN, B. B. (1954). The formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. *Exp. Cell. Res.* **7**, 558-562.
- HILLARP, H. (1946). Structure of the synapse and the peripheral innervation apparatus of the autonomic nervous system. *Acta Anat.* **2**, Suppl. 4, 1-153.
- NONIDEZ, J. F. (1944). The present status of the neurone theory. *Biol. Rev.* **19**, 30-40.
- PALADE, G. E. (1954). Electron microscope observations of interneuronal and neuromuscular synapses. *Anat. Rec.* **118**, 335-336.
- ROBERTSON, J. D. (1954). Electron microscope study of an invertebrate synapse. *Fed. Proc.* **13**, 119.
- ROBERTSON, J. D. (1955). Recent electron microscope observations on the ultrastructure of the crayfish median-to-motor giant synapse. *Exp. Cell Res.* **8**, 226-229.
- SJÖSTRAND, F. S. (1954). Synaptic structures of the retina of the mammalian eye. *Int. Conf. E.M. London*.
- STÖHR, P. JR. (1932). *Cytology and Cellular Pathology of the Nervous System*, ed. W. Penfield. New York: Hoeber.
- TIEGS, O. W. (1926). The structure of the neurone junctions of the spinal cord. *Aust. J. exp. Biol. med. Sci.* **3**, 69-79.
- WYCKOFF, R. W. G. & YOUNG, J. Z. (1954). The organization within neurons. *Proc. Anat. Soc. J. Anat., Lond.*, **88**, 568.









EXPLANATION OF PLATES

All the illustrations are electron micrographs of osmic fixed material. Cytoplasm of neurone, *neur.*; satellite neurone membrane, *s.n.*; cytoplasm of satellite cell, *sat.*

PLATE 1

- Fig. 1. Portion of a normal ganglion cell, showing two presynaptic axons. On the left, separated from the satellite neurone membrane (*s.n.*) by about 0.5μ , is an axon (*a.*) containing several mitochondria (*mi.*), and masses of small vesicles. Several layers of membrane (*m.*) separate the synaptic elements. Satellite cell cytoplasm (*sat.*) and neurone cytoplasm (*neur.*) are indicated. On the right is an axon (*a.*) much closer to the satellite neurone membrane. Concentric membranous bodies are seen at (*con.*).
- Fig. 2. Numerous presynaptic axons (*a.*) ending on a normal ganglion cell. The satellite neurone membrane (*s.n.*) between ganglion and satellite cell is indicated, the presynaptic axons are widely varied in size and composition—vesicles (*ves.*) and concentric bodies (*con.*) can also be seen.

PLATE 2

- Fig. 3. Region of a normal ganglion cell with liposomes (*l.*) close to the cell surface. A few presynaptic axons (*a.*) are indicated, widely separated from the satellite neurone membrane (*s.n.*), from which, at one point, an invagination (*in.*) arises, curling close to the axon-membrane. Other invaginations of the membranes may also be seen.
- Fig. 4. Portion of a normal ganglion cell, with several axons (*a.*). One of these is comparatively close to the satellite neurone membrane (*s.n.*) from which an invagination (*in.*) arises, curling close to the axon-membrane. Other invaginations of the membranes may also be seen.

PLATE 3

- Figs. 5, 6. Two sections through different parts of the same satellite cell hillock. The satellite neurone membrane (*s.n.*) is faintly indicated. Numerous axons (*a.*) are to be seen, one, shown in both pictures, has a second membrane around it (*a.s.*). The sections are separated by a small distance—as may be seen, the mitochondria in (*a.s.*) differ slightly in form in the two sections. The cytoplasmic membranes (*c.m.*) may be seen in many parts of the cell; they appear similar in the two sections suggesting that they are mostly in sheet form, but in a few places they appear in oval form, suggesting tube-like invaginations (*tu.*).

PLATE 4

- Fig. 7. A single axon (*a.*), rich in mitochondria (*mi.*), close to the ganglion cell; several layers of membrane (*m.*) lie between it and the satellite neurone membrane (*s.n.*).
- Fig. 8. Region of the neurone surface rich in presynaptic axons, some very close to the satellite neurone membrane, e.g. at *a.1* and *a.2*. The neuronal cytoplasm contains numerous large, irregular-shaped mitochondria, while several of the axons show fibrils (*f.*) a few vesicles (*ves.*) and mitochondria (*mi.*).
- Fig. 9. A concentric membrane whorl (*con.*) in the satellite cytoplasm near the neural and satellite junction (*s.n.*), this whorl is made up of concentric double membranes.
- Fig. 10. Region of satellite neurone junction, showing origin of the concentric body (*con.*). An invagination of the satellite neurone membrane (*s.n.*) makes two loops, then ends in a concentric body.
- Fig. 11. Satellite, neural region with a few Schwann cells 16 days after preganglionic section. The Schwann cells (*Sch.*) are deneurotized, but one mesaxon (*mes.*) with shrunken loop seems to have persisted.

THE DEVELOPMENT OF A COMPENSATORY COLLATERAL CIRCULATION TO NERVE TRUNK

By MICHAEL J. BLUNT* AND KATHLEEN STRATTON

Departments of Anatomy and Physics, Royal Free Hospital School of Medicine

Acute experiments have been reported in which it was demonstrated that the effective circulation to a nerve trunk may depend much more upon its regional vessels of supply than upon its intrinsic longitudinal plexuses (Blunt & Stratton, 1956).

In view of the results reported by Adams (1943) and Baesich & Wyburn (1945), it was considered that this initial predominance of the regional blood supply might be rapidly superseded after injury. To test the truth of this hypothesis a series of recovery experiments has been carried out, the results of which are here presented.

MATERIAL AND METHODS

Forty-two rabbits drawn from mixed breeds and from both sexes, with weights ranging from 1600 to 3020 g., formed the material for this investigation. The techniques used have already been described (Blunt & Stratton, 1956), and only details in which there has been some divergence from the original technique will be noted here.

In the present series the volume of Krebs-Ringer solution used to inject the tibial division of the sciatic nerve varied from 0.0004 to 0.005 ml. and its radioactivity varied from about 400 μ c./ml. to about 1.1 mc./ml. β counts of 30 sec. duration at 1 min. intervals were recorded over the injection site during two consecutive periods of 20 min., commencing 7 min. after completion of the injection. Between the two 20 min. periods an interval of from 1 to 4 min. permitted operative interference with the blood supply of the nerve. In no experiment did the rectal temperature vary by more than 0.5° C.

Control series

In twelve rabbits the vessels supplying the sciatic nerve at its lower end were under-run and ligated with fine ophthalmic sutures, each artery and companion vein together. The nerve was then injected with the radioactive Krebs-Ringer solution and counts were recorded during the first 20 min. period. At the conclusion of the period the terminal divisions of the sciatic nerve and the sural nerve were collectively and tightly ligated just above the knee, and counting was resumed for a second 20 min. period.

Survival series

The rabbits were anaesthetized with intraperitoneal Nembutal and ether, and the vessels to the lower end of the sciatic nerve were ligated. After survival periods of approximately 5, 10 and 20 days respectively, in groups 2, 3 and 4, the nerve was

* Present address: Department of Anatomy, Medical College of St Bartholomew's Hospital.

re-exposed under intravenous urethane anaesthesia and ^{24}Na clearance was studied, exactly as in the control experiments. In the case of the survival experiments, however, it was necessary to mobilize the nerve by dividing fine scar tissue around its lower end before commencing the clearance studies. The exact times of survival in individual experiments are shown in the tables.

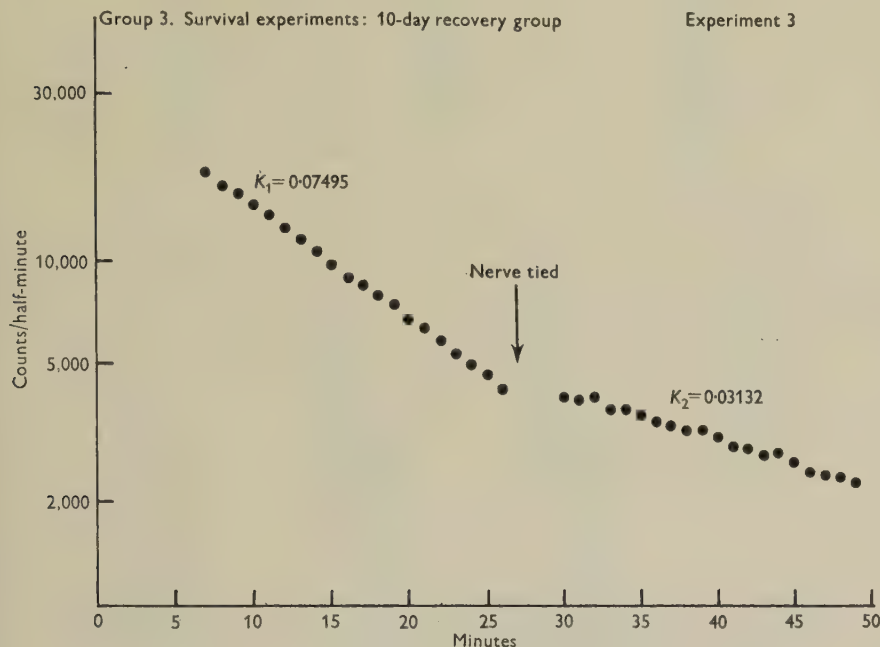


Fig. 1. A typical result from the 10-day group of experiments.

RESULTS

Group 1. Control experiments (Table 1)

In the control animals, wherein ligature of the regional vessels was followed later in the same experiment by nerve ligature, the alteration in the mean initial clearance slope for the whole group of experiments amounted to 30.00%.

Group 2. Five-day survival experiments (Table 2)

None of the ten animals comprising this group showed any macroscopic evidence of new regional vessel formation at the lower end of the nerve. The alteration in the mean initial clearance slope resulting from nerve ligature was 45.85%.

Group 3. Ten-day survival experiments (Table 3)

Three out of the ten animals in this group exhibited extremely fine collateral vessels to the nerve in the scar tissue at the lower end of the thigh, and division of the newly formed vessels was necessary before mobilization of the nerve, prior to its insulation from the surrounding tissue fluid. In the remaining seven animals no

Table 1. *Group 1. Survival experiments: control group*

Initial clearance slope: $ K_1 $	Final clearance slope: $ K_2 $	Slope change: $ K_1 - K_2 $
0.02210	0.01438	0.00772
0.03086*	0.01768	0.01300
0.05282	0.03287	0.01995
0.04793	0.02947	0.01846
0.04366	0.03620	0.00746
0.05056	0.02742	0.02314
0.05374	0.03502	0.01872
0.03993	0.03037	0.00956
0.03726	0.03132	0.00594
0.04796	0.03822	0.00974
0.04421	0.03122	0.01299
0.02521*	0.02310	0.00211
Mean 0.04134	—	0.01240

$$\frac{\overline{K_1 - K_2}}{\overline{K_1}} \times 100 = 30.00.$$

Twenty separate observations were used in the calculation of each parameter.

* Experiments omitted by random selection from covariance analysis to equalize numbers in groups.

Table 2. *Group 2. Survival experiments: 5-day recovery groups*

No. of days of recovery	Initial clearance slope: $ K_1 $	Final clearance slope: $ K_2 $	Slope change: $ K_1 - K_2 $
4	0.02881*	0.01919*	0.00962
6	0.02915	0.01082	0.01833
6	0.05344	0.02335	0.03009
5	0.02198	0.00933	0.01265
4	0.03422	0.01329	0.02093
4	0.02293	0.01738	0.00555
7	0.04829	0.02425	0.02404
6	0.02862	0.01767	0.01095
5	0.03182	0.02250	0.00932
5	0.02940	0.02018	0.00922
Mean	0.03287	—	0.01507

$$\frac{\overline{K_1 - K_2}}{\overline{K_1}} \times 100 = 45.85.$$

* Eighteen separate observations were used in the calculation of each parameter in this experiment. For the calculation of the parameters in remaining experiments twenty observations were used in each case.

Table 3. *Group 3. Survival experiments: 10-day recovery group*

No. of days of recovery	Initial clearance slope: $ K_1 $	Final clearance slope: $ K_2 $	Slope change: $ K_1 - K_2 $
12	0.07014	0.02905	0.04109
10	0.03935	0.01384	0.02551
12	0.07495	0.03132	0.04363
12	0.06224	0.02772	0.03452
10*	0.03459	0.01304	0.02155
11	0.02903	0.01339	0.01564
11*	0.03784	0.02768	0.01016
10	0.03596†	0.01675	0.01921
11	0.03762	0.01805	0.01957
11*	0.03698	0.01617	0.02081
Mean	0.04587	—	0.02517

$$\frac{\overline{K_1 - K_2}}{\overline{K_1}} \times 100 = 54.87.$$

* Fine collateral vessels had developed in these experiments.

† Fourteen separate observations used in the calculation of this parameter. Twenty observations used for each of the other parameters shown.

such collateral vascular pathways were encountered. Nerve ligature produced a mean percentage clearance slope alteration of 54.87. In only one of the three animals with new collateral vessels was the proportionate alteration in clearance slope less than the mean clearance slope alteration for the whole of group 3.

Group 4. Twenty-day survival experiments (Table 4)

In seven of the ten animals in this group new, well-formed regional vessels were present in the scar tissue around the lower end of the nerve. These collateral vessels by-passed the ligatures on the original vessels and joined the longitudinal epineurial vessels of the nerve trunk, and their division was essential to mobilization thereof.

Table 4. *Group 4. Survival experiments: 20-day recovery group*

No. of days of recovery	Initial clearance slope: $ K_1 $	Final clearance slope: $ K_2 $	Slope change: $ K_1 - K_2 $
20	0.06390	0.04827	0.01563
20	0.03589	0.01609	0.01980
21*	0.03748	0.01389	0.02359
21	0.03954	0.02506	0.01448
22	0.05031	0.03562	0.01469
22	0.04937	0.02805	0.02132
21	0.04271	0.03426	0.00845
21*	0.05557	0.03086	0.02471
19	0.04499	0.02344	0.02155
18*	0.06573	0.04342	0.02231
Mean	0.04855	—	0.01865

$$\frac{K_1 - K_2}{K_1} \times 100 = 38.41.$$

Twenty separate observations were used in the calculation of each parameter.

* No collateral vessels were evident in these experiments.

In the three animals in which new regional vessels were not observed, their possible presence could not be definitely excluded since small vessels might have remained hidden in the scar tissue which was here considerably denser than in the earlier groups of survival animals. Nerve ligature produced a mean percentage slope alteration of 38.41.

DISCUSSION

Techniques

The validity of the ^{24}Na clearance technique employed has been discussed previously (Blunt & Stratton, 1956). Covariance analysis was used to study the possible differences between slope alterations in different groups of experiments, and revealed significant differences between the control and the first two survival groups, but not between the control and the 20-day group of experiments.

Circulatory alterations

The clearance slope alteration obtained experimentally for the control group of these recovery experiments was $\frac{\overline{K_1} - \overline{K_2}}{\overline{K_1}} = 30.0\%$. (The subscripts v and n refer to ligature of the vasa nervorum and the nerve respectively.)

On the basis of data from the acute experiments previously reported, an estimate of the value to be expected can be made as follows: a slope alteration $\frac{\overline{K_1} - \overline{nK_2}}{\overline{K_1}} = 53.74\%$ followed nerve ligation, and an alteration of $\frac{\overline{K_1} - \overline{vK_2}}{\overline{K_1}} = 48.8\%$ followed ligation of the vasa nervorum at the lower end of the thigh, in the acute experiments. Using these data alone a clearance slope reduction $\frac{\overline{vK_2} - \overline{nK_2}}{\overline{vK_2}}$ of the order of 9.6% might be expected in the control series of the present recovery experiments. There was, however, a clearance slope alteration $\frac{\overline{K_1} - \overline{K_2}}{\overline{K_1}} = 16.82\%$ in the control series of the acute experiments where no vascular interruption was undertaken, and it was concluded that this was probably due to the fact that two different exponentials are involved in the clearance of radioactive sodium from mammalian nerve trunk (Dainty & Krnjević, 1955), and also, possibly, to a gradual progressive alteration in the exposed vascular bed. Such considerations are also applicable to the present recovery series of experiments, so that the value of $\overline{vK_2}$ used in the above estimate of 9.6% for the control group slope reduction is too small because it was determined during the *second* 20 min. counting periods of the acute experiments. If a correction is made for this, it is estimated that a slope reduction $\frac{\overline{vK_1} - \overline{nK_2}}{\overline{vK_1}} \div 25\%$ should be obtained in the recovery control group. This compares with the direct experimental value of 30%. In the survival experiments, a comparable result would indicate that no compensatory vascular change had taken place after the original ligation of regional vessels. On the other hand, it was found from the acute experiments that nerve ligation alone, at the lower end of the thigh, produced a clearance slope alteration of 53.74%. A result similar to this, and obtained in the survival experiments of the present series, would indicate that complete vascular compensation had occurred.

The 45.85% reduction in clearance slope in the 5-day survival experiments was significantly different from the alteration obtained in the controls, and indicated that substantial vascular compensation had taken place. Since no new regional vessels were macroscopically evident in the animals of this group of experiments, the compensation must have been effected by way of the intrinsic longitudinal plexuses of the nerve trunk. The increased relative importance of these plexuses was reflected in the greater clearance slope alteration which followed their occlusion in the first group of survival experiments (group 2). That the plexuses were effectively occluded by the ligatures was shown from the earlier acute experiments.

In the group 3 experiments (10 days survival) a mean percentage clearance slope alteration of 54.87 followed nerve ligation. While not significantly different from the results in the 5-day group, this result is closely similar to the alteration of 53.74% which would be expected if full vascular compensation had been established. In the three experiments in which it was necessary to divide newly formed regional vessels, the results were not significantly different from those in the rest of the group. It may therefore be concluded that, by the end of 10 days, vascular compensation,

complete under the conditions of the experiments, had taken place by means of the intrinsic longitudinal plexuses of the nerve trunk.

In the group 4 experiments (20 days survival) well-defined, newly formed collateral channels to the lower end of the nerve were divided in all but three animals. The mean percentage clearance slope alteration of 38.41 which followed ligation of the nerve was higher than that obtained in the control series, but the difference was not significant. This finding, and the appearance of the newly formed regional vessels, would suggest that the vascular arrangements were comparable with those of the normal animals. Further support for this deduction comes from the fact that the results in the group 4 experiments differed significantly from those in group 3. It may be concluded that the new regional vessels which were divided at the beginning of the group 4 experiments had substantially assumed the importance of their original predecessors. In the three animals in which collateral vessels were not evident the results were not significantly different from those in the rest of the group, although two of them indicated, as would be expected, somewhat higher proportionate clearance slope alterations than the mean clearance slope alteration for the whole of group 4.

All the survival experiments therefore show a striking early compensation for the effects of ligation of segmental vessels. The compensation took place at first by way of the intrinsic longitudinal plexuses of the nerve trunk, and it was complete, in the conditions of the experiments, by the end of 10 days. Estimations of ^{24}Na clearance have, however, been made only in animals with the limb at rest under anaesthesia, and it seems likely that a small deficiency in the vascular arrangements still remained and provided the stimulus to the growth of the new regional vessels, which were a feature of the 20-day survival experiments.

SUMMARY

In the recovery experiments reported there was a striking early compensation for the effects of ligation of the segmental vessels to the lower end of the rabbit sciatic nerve. This compensation was effected by the intrinsic longitudinal plexuses of the nerve and was complete, in the conditions of the experiments, by the end of 10 days.

Despite this early recovery, new growth of regional vessels took place, and by the end of 20 days they made a substantial contribution to the blood supply of the nerve on a segmental basis.

The authors wish to acknowledge their gratitude to Dr D. A. Sholl who has advised on the statistical treatment of the data, to Prof. R. E. M. Bowden and Dr H. A. B. Simons for their helpful interest in this research and for criticizing the manuscript, and to Prof. A. J. E. Cave for criticizing the manuscript. Mr H. S. Williams has kindly dispensed all the ^{24}Na in a form suitable for use in the experiments. Mr G. Champion has given us helpful technical assistance.

REFERENCES

- ADAMS, W. E. (1943). The blood supply of nerves. *J. Anat., Lond.*, **77**, 243-250.
- BACSICH, P. & WYBURN, G. M. (1945). The effect of interference with the blood supply on the regeneration of peripheral nerves. *J. Anat., Lond.*, **79**, 74-82.
- BLUNT, M. J. & STRATTON, K. (1956). The immediate effects of ligature of vasa nervorum. *J. Anat., Lond.*, **90**, 204-216.
- DAINTY, J. & KRNJEVIC, K. (1955). The rate of exchange of ^{24}Na in cat nerves. *J. Physiol.* **128**, 489-503.

THE ORGAN OF JACOBSON

BY V. E. NEGUS

Ferens Institute, Middlesex Hospital, London

Although 250 years have passed since Ruysch (1703) described the structure now known as the organ of Jacobson (1811), the considerable volume of work devoted to the subject since then has not led to definite conclusions as to the organ's function. The bilateral organ in question is so beautifully designed that one cannot fail to ascribe a purposive function to it; it seems incredible that a carefully arranged system of specialized epithelium, with its own nerve supply, as described by Elliot Smith (1897), McCotter (1917) and others, with numerous glands emptying into it and with a duct communicating with the exterior by a more or less devious route, should not play some important part in the animal economy.

The object of this paper is, in part, to present certain observations on the anatomy of the organ in different groups of animals, but primarily to put forward ideas with regard to its possible role during life. The bibliography is extensive, but amongst general references are Klein (1880), Read (1908), Pearlman (1934), Romer (1949) and Allison (1953).

MATERIAL AND METHODS

The nasal region has been studied in the following animals: newt (*Triturus vulgaris*), toad (*Bufo bufo*), frog (*Rana temporaria*), slow worm (*Anguis fragilis*), guinea-pig (*Cavia porcellus*), rabbit (*Lepus cuniculus*), cat (*Felis domestica*) and dog (*Canis familiaris*).

Following intravital fixation and subsequent decalcification, serial sections were cut, of which every first, fifth, tenth or twentieth was stained, according to the complexity of structural detail. To supplement microscopic observation transparent reconstructions of the nose were prepared. In this way the nasal organ was reproduced as a three-dimensional model and studied either as a whole, or in parts, comprising as many sections as desired. The method of making the models was simply that of photographing the sections as negatives, which were then enlarged as half-plate or whole-plate positives according to size. Histological details were selectively tinted, the plates were then mounted serially in a rack and examined by transmitted illumination.

FINDINGS

Newt

A small lateral recess lined by epithelium of olfactory type, distinct from that of the nasal fossa itself, is present on each side. Communication with the exterior is indirect, via the nasal fossae and the anterior and posterior choanae. It is possible that this simple recess serves to retain odorous substances so that an after-smell is perceived when the air current has removed any olfactory substance from the nasal fossa itself; it would thus discharge a function assumed in other species by the specialized organ. The organ in various Amphibia was described by Seydel (1895).

Toad and frog

In these amphibians a separate recess, in the form of a tubular passage lined by olfactory epithelium, is found in communication with the nasal fossa through a narrow duct about 13.5μ in diameter (Pl. 1, fig. 1).^{*} Connexion with the exterior is indirect, via the nasal fossa and through the anterior and posterior nares. Here again retention of odorous molecules may serve a useful purpose; it is clear, however, that with such a narrow channel of communication the volume of scent-laden air which can reach the organ of Jacobson must be very small, and its rate of passage very slow.

Slow worm

Amongst reptiles no distinct and isolated organ of Jacobson is found in the Crocodilia, nor in the Chelonia—tortoises and turtles (McCotter, 1917). Snakes and lizards are, however, so provided, but in them the organ opens directly on to the palate, without any communication with the nasal fossa (Pl. 1, fig. 2).

It has been reported that these reptiles touch substances in their path with their pronged tongues, the tips of which are then placed near the ducts of Jacobson's organ (Pratt, 1948). The nature of the object touched would then be perceived by the olfactory sense. However, the duct by my measurement is only 13.5μ in diameter (see footnote) and such a small size would necessitate extreme accuracy of tongue movement for any useful information to be derived. The epithelium, which is approximately 95μ in thickness, is symmetrically arranged in a concave crescentic form and appears to be of usual olfactory type. On the opposite wall of the organ the epithelium is ciliated and is about 15μ in thickness.

Mammals

The rabbit has been examined as a member of the order Lagomorpha. It has a beautifully arranged organ of Jacobson, paired and symmetrical. The epithelium is similar to that of the foregoing classes and its nerves are big and well defined; they are connected to the accessory olfactory bulb (Adrian, 1954). The duct from the tubular organ opens by a pore into the lower part of the nasal fossa; it is $27-31.5\mu$ in diameter (Pl. 1, fig. 3). As in the Amphibia referred to above, communication with the exterior is indirect; it is, in the rabbit, into the nasal fossa, at some little distance from the nasal opening of the naso-palatine canal. Communication with the mouth is therefore indirect. The guinea-pig has a similar arrangement. Retention of after-smells would once again appear to be probable.

A well-marked organ is found in Carnivora; reconstructions of *Felis*, the domestic cat, and *Canis familiaris*, the domestic dog, have provided the opportunity for a clear study.

The structure of the organ is much like that of the rabbit, but its method of communication differs. Examination of consecutive serial sections shows that the organ opens directly into the naso-palatine canal (Pl. 1, fig. 4). The duct leading

^{*} All measurements given are from prepared specimens after dehydration and embedding in celloidin. They are useful for comparison, but do not necessarily represent the state during life. For instance, the palatal opening of the naso-palatine canal in a rabbit will admit a tube of 700μ diameter, owing to stretching of the tissues.

from the anterior end of the organ in the cat is $30-40\mu$ or rather more in diameter and the opening on to the palate is about 50μ .

It is found, therefore, that there are four types of communication: the first, as in *Amphibia*, opens into the nasal fossa; the second, as in the snake and lizard, has no connexion with the nasal fossa but opens directly on to the palate; the third, in the rabbit and guinea-pig, opens into the nasal fossa which itself is connected with the mouth by the naso-palatine canal; and the fourth, as in *Carnivora*, communicates both with the nasal fossa and with the mouth by opening into the naso-palatine canal.

The study of reconstructions and the measurement of the area of mucosa of olfactory type in Jacobson's organ give a rough estimate of relative areas in different animals. It appears that the actual surface area in the slow worm is less than that of the rabbit and still less than that of the cat.

On the other hand, a comparison of the olfactory areas in the organ of Jacobson and in the nasal fossa of mammals reveals a vast proportional increase of the latter as compared with the former.

The deduction is that while Jacobson's organ retains a position of importance in the mammals examined, yet the enormous increase of olfactory mucosa in their nasal fossae indicates a greatly increased reliance on olfaction in the nose as compared with the accessory organ.

The question of transference of odours to the olfactory region of Jacobson's organ presents considerable difficulty. Thus in the toad the communication between the organ and the nasal fossa is only 13.5μ in diameter. This would make transference of air by diffusion a slow process.

Again, in the slow worm the palatal duct is still no more than 13.5μ in diameter; there are no glands in the lumen of the organ, but cilia are present on the prominence facing the olfactory area (Pl. 1, fig. 2); and if these cilia were to carry in odorous secretion, it is difficult to see how it would be expelled.

In mammals the palatal duct is lined by thick squamous epithelium devoid of cilia (Pl. 1, fig. 4); in the olfactory part of the organ cilia are present, no doubt to expel secretion derived from the glands of Jacobson. These cilia were described by Kolliker (1877) and Mihalovics (1899).

It has been suggested that odorous secretions are forced upwards in those species where the duct opens through the palate; this would be possible in the cat or dog by pressure on the mushroom-shaped pad between the two orifices (Pl. 2, fig. 5), but it would only serve to propel a little fluid up the naso-palatine canal into the nasal fossa, since the organ of Jacobson is an air-locked tubule communicating laterally with the canal (Pl. 1, fig. 4). This observation can be confirmed experimentally. It would appear probable that the pad serves to prevent entrance of fluid.

It seems likely that olfactory molecules are carried in air rather than in fluid, and that a pumping action of the vascular spaces at the posterior end of Jacobson's organ draws in or expels air by alternate emptying and filling of these blood sinuses, as described by Hamlin (1930); the spaces are well seen in the guinea-pig and in the rabbit (Pl. 2, figs. 6, 7). Vascular spaces are absent in the slow worm (Pl. 2, fig. 8). The pump action was referred to by Broman (1920), but he thought that fluid and not air was the vehicle; Seydel (1895) was of the same opinion.

If the question of retention of olfactory molecules to give an after-smell is considered in relation to anatomical structure, it will be relevant to observe that in both the slow worm and toad there is no recessing of olfactory mucosa. The nasal fossae are more or less circular passages lined in part by specialized epithelium, and any olfactory molecules brought in through the anterior nares will pass down through the posterior nares unless they adhere to the epithelial surfaces. Consequently, when respiration is active, the exchange of air will be complete.

In Carnivora, on the other hand, there is very considerable recessing, a great part of the olfactory mucosa being tucked away in frontal or sphenoidal sinuses, or placed out of the direct respiratory stream above a sub-ethmoidal plate. After-smells will thus be retained, and the role of the organ of Jacobson as an after-smell device will thereby be rendered less important.

Why the organ should communicate with the nose alone in some animals and with the nose and mouth in others is less easy to explain.

SUMMARY AND CONCLUSIONS

1. Although the olfactory sense has progressed to an advanced state of acuity and discrimination in mammals, the organ of Jacobson is nevertheless retained and is of functional importance.

2. On anatomical grounds it appears probable that air and not fluid enters the tubule, and that it conveys odours or flavours connected with the diet of the animal.

3. The duct of Jacobson's organ is of such extreme narrowness, both relative to its length and absolutely, that the passage of air into it must take place slowly.

4. The most likely function of the organ would seem to be related to the after-smell of food rather than to its immediate detection. An associated role in connexion with the secretion of gastric juice is possible.

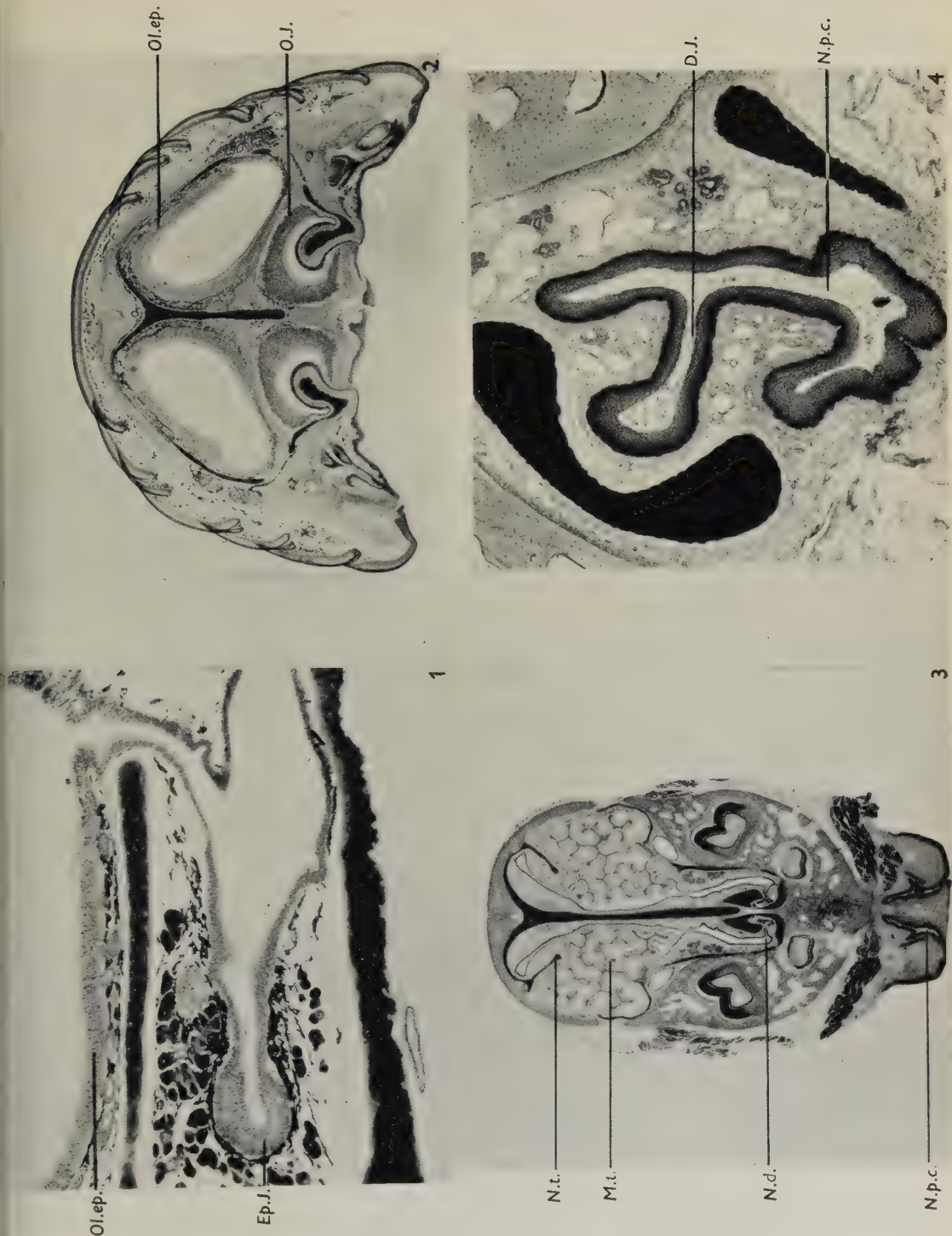
I am indebted to Prof. Walls for valuable suggestions in the preparation of this communication. Prof. Boyd and Dr Bellairs have kindly given advice. My son has given help in the study of the organ, and Mr J. C. Seymour has been asked for criticism.

The cutting of serial sections and a great part of the making of reconstruction models has been carried out by Mr D. Bishop, chief technician to the Ferens Institute; preparation of the positive enlargements has been in the hands of Mr Turney, of the Photographic Department of the Middlesex Hospital.

The facilities of the Ferens Institute and of the Royal College of Surgeons have been of great benefit.

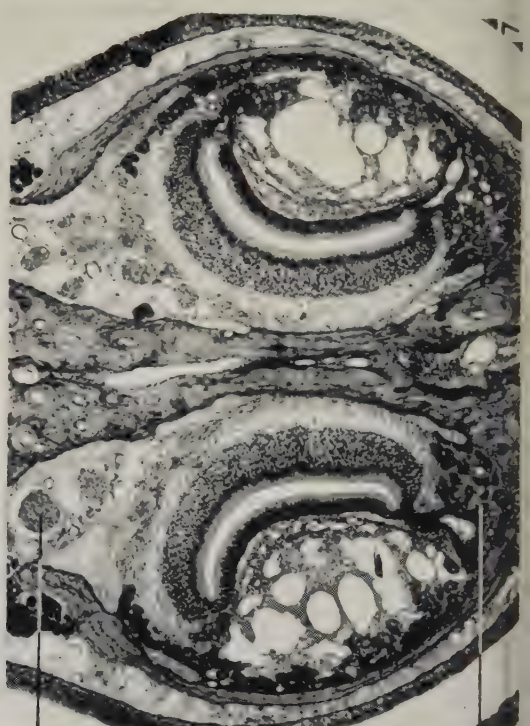
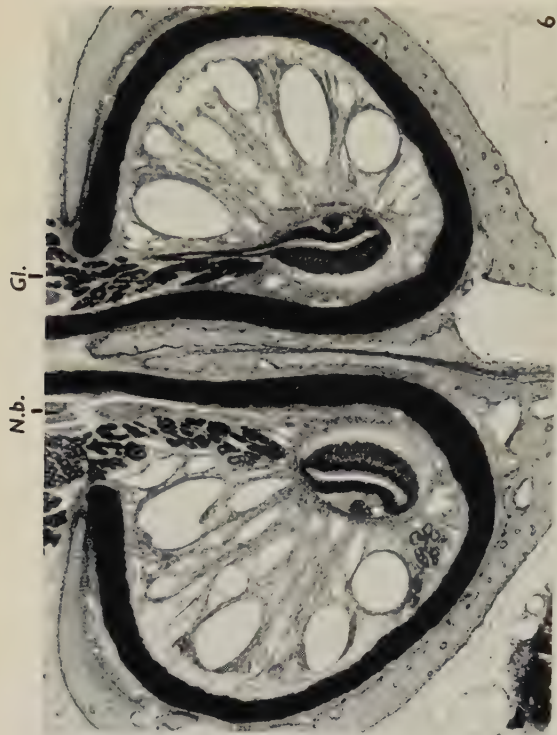
REFERENCES

- ADRIAN, E. D. (1954). Synchronized discharges from the organ of Jacobson. *J. Physiol.* **126**, 28 P.
- ALLISON, A. C. (1953). The morphology of the olfactory system in the vertebrates. *Biol. Rev.* **28**, 195-244.
- BROMAN, I. (1920). Das Organon Vomero-nasale Jacobsoni—ein Wassergeruchorgane. *Anat. Hefte*, **58**, 137-191.
- ELLIOT SMITH, G. (1897). The brain of a foetal *Ornithorhynchus*. *Quart. J. micr. Sci.* **39**, 181-206.
- HAMLIN, H. E. (1930). Working mechanisms for the liquid and gaseous intake and output of Jacobson's organ. *Amer. J. Physiol.* **91**, 201-205.



NEGUS—THE ORGAN OF JACOBSON

(Facing p. 518)



- JACOBSON, L. (1811). Description anatomique d'un organe observé dans les Mammifères. *Ann. Mus. Hist. nat. Paris*, 18, 412-424.
- KLEIN, E. (1880). A contribution to the minute anatomy of the organ of Jacobson. *St Bart's Hosp. Rep.* 16, 1-7.
- KOLLIKER, A. (1877). Über die Jacobson'schen Organe des Menschen. In Von Reinecke, F., *Festschrift*, 4, 1-12. Leipzig.
- MCCOTTER, R. E. (1917). The vomero-nasal apparatus in *Chrysemys punctata* and *Rana catesbiana*. *Anat. Rec.* 13, 51-67.
- MIHALOVICS, VON (1899). Nasenhöhle und Jacobsonsches Organ. *Anat. Hefte*, 1 (Abt. II), 1-108.
- PEARLMAN, S. J. (1934). Jacobson's Organ (Organon Vomero-Nasale Jacobsoni): Its anatomy, gross, microscopic and comparative, with some observations as well on its function. *Ann. Otol. Rhinol. Laryngol.* 43, 739-768.
- PRATT, C. W. McE. (1948). The morphology of the ethmoidal region of sphenodon and lizards. *Proc. Zool. Soc. Lond.* 118, 171-201.
- READ, E. A. (1908). A contribution to the knowledge of the olfactory apparatus in dog, cat and man. *Amer. J. Anat.* 8, 17-47.
- ROMER, A. S. (1949). *The Vertebrate Body*. Philadelphia and London: W. B. Saunders.
- RUYSCH, F. (1703). *Thesaurus Anatomicus*. Vol. III, p. 49. Amsterdam, Wolters.
- SEYDEL, O. (1895). Über die Nasenhöhle und das Jacobson'sche Organ der Amphibien. *Morph. Jb.* 23, 453-543.

KEY TO ABBREVIATIONS

<i>D.J.</i>	Duct of Jacobson's Organ.	<i>N.d.</i>	Nasal duct.
<i>Ep.J.</i>	Epithelium of Jacobson's Organ.	<i>N.p.c.</i>	Nasopalatine canal.
<i>Gl.</i>	Multicellular glands.	<i>N.t.</i>	Naso-turbinal.
<i>M.t.</i>	Maxillo-turbinal.	<i>Ol.ep.</i>	Olfactory epithelium of nasal fossa.
<i>N.b.</i>	Nerve bundles.	<i>O.J.</i>	Organ of Jacobson.

EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Toad (*Bufo bufo*). The nasal fossa above communicates by a narrow channel with the organ of Jacobson below; the specialized epithelium of the latter is thicker than that of the former.
- Fig. 2. Slow worm (*Anguis fragilis*). Transverse section to show the relative sizes of the nasal fossae and the paired organ of Jacobson; the epithelial lining of the latter is thick.
- Fig. 3. Rabbit (*Lepus cuniculus*), showing the nasal duct of Jacobson's organ and also the commencement of the nasopalatine canal.
- Fig. 4. Cat (*Felis domestica*). The duct of Jacobson opens into the medial wall of the nasopalatine canal; both are lined by squamous epithelium.

PLATE 2

- Fig. 5. Dog (*Canis familiaris*). The orifices of the two nasopalatine canals are protected by a mushroom-shaped flap.
- Fig. 6. Rabbit (*Lepus cuniculus*). The olfactory part of the organ is contained in a cartilaginous capsule, within which are wide vascular spaces.
- Fig. 7. Guinea-pig (*Cavia porcellus*). The olfactory part of the organ lies within a bony capsule and has wide vascular spaces on its outer side.
- Fig. 8. Slow worm (*Anguis fragilis*). Posterior end of the organ with cells of the specialized epithelium cut across. There are no vascular spaces.

THE OCCURRENCE OF A MIDDLE SUPERIOR ALVEOLAR NERVE IN MAN

By M. J. T. FITZGERALD

Department of Anatomy, University College, Cork

The observations of Wood Jones (1939) led him to deny the common occurrence of a middle superior alveolar nerve leaving the infra-orbital groove to join the alveolar plexus above the premolar teeth. The descriptions of the superior alveolar nerves in British text-books are largely based on Wood Jones's account.

The routine dissections of the alveolar nerves made in this department have not conformed to the newer accounts. Consequently a further investigation was made and the results appear worth recording. In the account which follows, the term 'middle superior alveolar nerve' will not be confined to a nerve arising in the infra-orbital groove. The criteria of definition are: (1) that it is intermediate in position between an anterior and a posterior nerve, and (2) that it joins the premolar alveolar plexus.

MATERIAL AND METHODS

The disposition of the superior alveolar nerves in the roof and walls of the antrum of Highmore was studied in fifty consecutive dissecting-room specimens obtained from twenty-eight human subjects. The following technique proved very satisfactory in practice: the external surfaces of the maxilla were exposed and the nasal wall of the antrum was completely removed. Illumination from its nasal aspect of the chamber so exposed did not reveal the nerves in its wall, but, unless the overlying mucous membrane were very thick, transillumination *ab externo* by a strong beam of light threw them clearly into silhouette. Under transillumination the mucous membrane was peeled downwards from the roof and lateral wall, care being taken that the nerves were not pulled off by traction on their mucosal branches. Finally, the infra-orbital canal was opened from above and the nerves were traced to their points of origin. Confirmatory histological examination of the middle alveolar nerves was also made.

Fifty dried maxillary bones (twenty-eight being from skeletal pairs) were examined and the disposition of their bony markings noted.

RESULTS

The various types of middle alveolar nerve which were found are illustrated in Fig. 1, and their relative incidence is shown in Table 1. Type 1 is an instance of premature origin of the nerve from the maxillary trunk; it pierces the bone a few millimetres lateral to or below the commencement of the infra-orbital groove. Types 2-4 take origin from the infra-orbital nerve and run in the posterior, lateral and anterior walls of the antrum respectively. In these instances the bony canal runs obliquely for 1-2 cm. from the lateral side of the infra-orbital canal, to be

succeeded by a groove which is directed towards the premolar alveolar plexus. Type 5 could be described as an instance of 'delayed separation' of the middle alveolar nerve bundle when we view it in relation to the other four types.

In no dissections were blood vessels seen in the absence of a nerve trunk; an appropriate bony marking on a dried maxilla was therefore accepted as the former

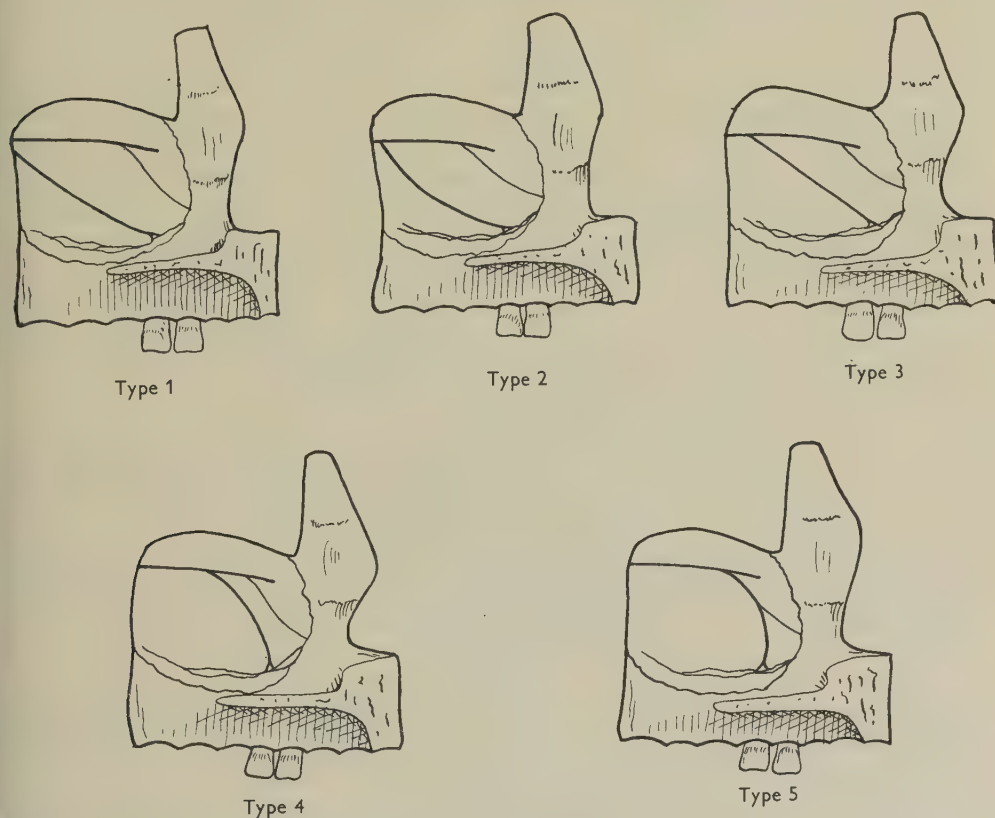


Fig. 1. Scheme of the modes of presentation of the middle superior alveolar nerve.

Table 1. *Incidence of the various types of middle superior alveolar nerve*

Nerve type	Dissected parts		Dried bones		Total
	No.	Origin of bony canal* (mm.)	No.	Origin of bony canal* (mm.)	
1	5	—	6	—	11
2	6	2	8	2	14
3	7	4	8	4	15
4	13	7.5	12	8	25
5	12	—	5	—	17
Absent	7	—	11	—	18
Total	50	—	50	—	100

* Mean distance from posterior end of infra-orbital groove.

site of a nerve trunk. This assumption appears to be justified by the remarkable agreement between the distribution of the bony canals in the dissections and those in the dried bones (Table 1).

DISCUSSION

Wood Jones's paper dealt primarily with the anterior superior alveolar nerve; but his statement that he could find no evidence of a middle nerve implies that the place of such a nerve is taken by branches of the anterior or posterior alveolar nerves, or both. The evidence given above does not support this implication. In this connexion, concurrent observations on the other alveolar nerves are relevant.

The anterior superior nerve is joined above the canine root by the forward continuation of the alveolar plexus. In four dissections the middle alveolar nerve received a fine but distinct branch from the anterior alveolar. This can be regarded as a combination of normal and delayed separation of the middle alveolar nerve or as an instance of overlap between the middle and anterior nerves.

The posterior alveolar nerves were found to form a loose peri-arterial bundle around the posterior superior alveolar artery, and could be traced along the course of this vessel as far as the premolar teeth; in which region the anterior and posterior alveolar arteries formed an arterial arcade.

It would seem, then, that the normal nerve supply of the premolar teeth and of the parts in their neighbourhood has a middle alveolar nerve as its primary (or central) stem. As elsewhere in the body, however, there is an overlap by nerve fibres carried in other trunks. Occasionally the central trunk is absent, and the channels of overlap take its place as the definitive nerve supply of these parts. In all this there is nothing morphologically different from what is true of other parts of the body.

SUMMARY

1. The course and distribution of the superior alveolar nerves were examined in fifty adult specimens, forty-four of which were pairs. Fifty dried bones were also examined, twenty-eight being pairs.

2. Evidence of a middle alveolar nerve arising from the infra-orbital trunk was found in 54 % of cases. Manifest variations of such a nerve were found in 28 % of cases. No evidence of a middle alveolar nerve could be found in the remaining 18 %.

3. The middle superior alveolar nerve may run in the posterior or lateral or anterior wall of the maxillary antrum.

4. The middle alveolar nerve passes to the premolar part of the alveolar plexus. Evidence was found of overlap between the distribution of this nerve and of the anterior and posterior alveolar nerves.

It is a pleasure to record my gratitude to Prof. M. A. MacConaill for his helpful advice during the construction of this paper. I wish to thank Prof. E. Keenan of the Anatomy Department, University College, Dublin, for the use of all his available material.

REFERENCE

- WOOD JONES, F. (1939). The anterior superior alveolar nerve and vessels. *J. Anat., Lond.*, **73**, 583-591.

THE ARRANGEMENT OF THE ANSA SPIRALIS OF THE OX COLON

By R. N. SMITH* AND G. W. MEADOWS†

The University, Bristol

The colon primum of the ox (homologous with the ascending colon of man) can be arbitrarily divided into three regions (Figs. 1, 2). The first, the ansa proximalis, is arranged in sigmoid fashion continuing cranially from the caeco-colic junction. It is continued by the second region, the ansa spiralis. This is usually an arrangement of centripetal and centrifugal coils forming a regular spiral. The third part,

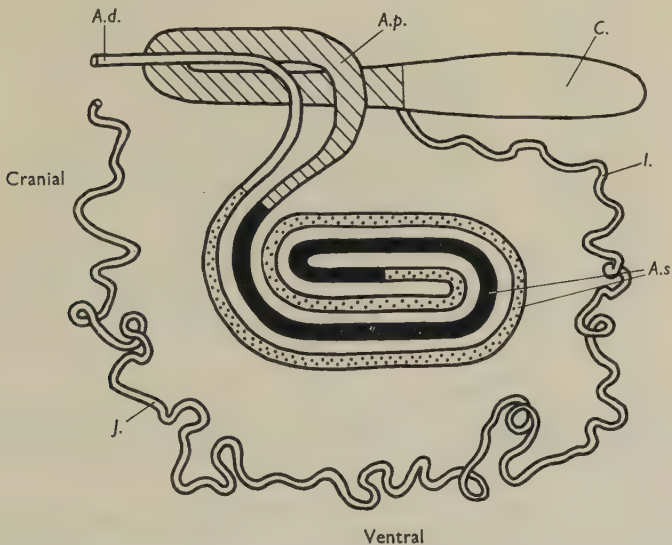


Fig. 1. Diagram of the arrangement of part of the alimentary tract of an ox, with an ansa spiralis (A.s.) having one and a half centripetal coils. A.d., ansa distalis; A.p., ansa proximalis; A.s., ansa spiralis (centripetal coils solid, centrifugal coils dotted); C., caecum; I., ileum; J., jejunum.

the ansa distalis, is a J-shaped loop which is continued by the colon secundum (homologous with the transverse colon of man). The ansa spiralis is firmly fixed to the common mesentery of the jejunum and ileum which are lying in an arc on the circumference. Martin & Schauder (1938) and Ackerknecht (1943) give the number of centripetal coils as one and a half to two. They also comment that irregularities sometimes occur in the coiling.

This survey was conducted to record the frequency of the different patterns of the ansa spiralis that occurred in a large number of cattle.

* Department of Veterinary Anatomy.

† Veterinary student.

MATERIALS AND METHODS

Seven hundred and thirty-five sets of ox intestines were examined within 6 hr. of slaughter. The ansa spiralis was considered to start and end at points level with the dorsal limit of the coil (see Figs. 1 and 2). The centripetal coils were counted to the centre of the spiral and the centrifugal coils from this point to the end of the ansa spiralis. This results in a half more centrifugal than centripetal coils. Fig. 1 is a diagram of part of the alimentary tract showing one and a half centripetal and two centrifugal coils in the ansa spiralis. Fig. 2 shows two centripetal and

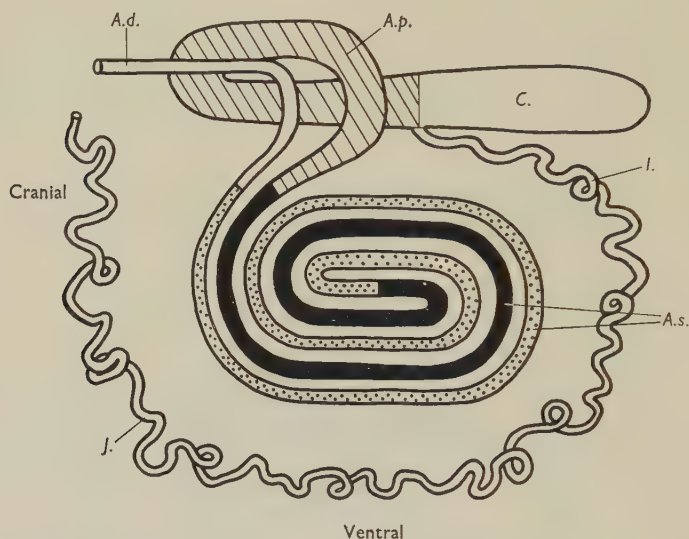


Fig. 2. Diagram of the arrangement of part of the alimentary tract of an ox, with an ansa spiralis (*A.s.*) having two centripetal coils. Abbreviations as for Fig. 1.

two and a half centrifugal coils. Although absolute bowel length might alter after death, the nature of the peritoneal fixation of the ansa spiralis precludes any change in the pattern. The breeds of the animals were not recorded, but it is known that many different ones were included in the survey.

RESULTS

The specimens could be divided into those showing a regular spiral and others in which there was some irregularity.

Regular spirals

Specimens occurred with the following numbers of centripetal coils: one (Fig. 3A), one and a quarter (Fig. 3B), one and a half (Fig. 1), one and three-quarters (Fig. 3C), two (Fig. 2), two and a quarter (Fig. 3D), two and a half (Fig. 3E).

The frequency of these patterns is shown in Table 1.

Irregular patterns

Twenty-four specimens were seen with irregular patterns. Some of these are shown in Fig. 4; of these two patterns occurred twice.



Fig. 3. Diagram to show some of the regular patterns of the ansa spiralis; centripetal coils solid, centrifugal coils dotted. A, one centripetal coil; B, one and a quarter centripetal coils; C, one and three-quarters centripetal coils; D, two and a quarter centripetal coils; E, two and a half centripetal coils.

Table 1. *The specimens with regular spiral patterns divided into groups according to the number of centripetal coils in the ansa spiralis*

No. of centripetal coils	No. of specimens	Percentage of total examined
1	1	0.14
1 $\frac{1}{4}$	1	0.14
1 $\frac{1}{2}$	179	24.35
1 $\frac{3}{4}$	7	0.95
2	520	70.75
2 $\frac{1}{4}$	1	0.14
2 $\frac{1}{2}$	2	0.27
Total	711	96.74



Fig. 4. Diagram showing some of the irregular arrangements of the ansa spiralis seen in the survey.

DISCUSSION

The survey shows that regular spirals occur in about 97% of cattle. Of these the commonest pattern is that with two centripetal coils which appears in 71% of those examined; those cattle with one and a half centripetal coils accounted for about 24%. The proportion of regular spirals is larger than is found in sheep.

Smith (1955*a*) gives a figure of approximately 80 % for regular patterns in that species. This may well be connected with the relatively simpler spiral of the ox; here the number of centripetal coils ranges from one to two and a half, whereas in the sheep the range is from two to four.

It is also interesting to note that in sheep about 25 % of the irregularities occurred in the last centrifugal coil (Smith, 1955*b*). This coil in the sheep is displaced from the remainder of the spiral, being separated by a broad band of common mesentery in which lymph nodes are embedded. In the ox, the last centrifugal coil is very close to the rest of the spiral and separated from the jejunum and ileum. No irregularities were seen in this coil in the ox.

SUMMARY

1. A brief description is given of part of the alimentary tract of the ox with especial reference to the colon primum (homologous with the ascending colon of man).
2. An analysis of the ansa spiralis of 735 cattle showed that 711 or 96·7 % had a regular spiral. Approximately 71 % had two centripetal coils and 24 % had one and a half centripetal coils.
3. Some of the irregular patterns are shown in diagram form.
4. The results are compared with those from a survey of the similar structure in sheep.

Our thanks are due to Mr L. M. King for his help in collecting some of the data, and to Miss B. Vowles for her assistance in the preparation of the manuscript. We would also like to thank Prof. C. W. Ottaway, in whose Department this work was done.

REFERENCES

- ACKERKNECHT, E. (1943). *Das Eingeweidesystem*. In Ellenberger-Baum, *Handbuch der vergleichenden Anatomie der Haustiere*, 18th ed. Berlin: Springer.
- MARTIN, P. & SCHAUDER, W. (1938). *Lehrbuch der Anatomie der Haustiere*, Bd. 3. *Anatomie der Hauswiederkäuer*, 3rd ed. Stuttgart: Schickhardt und Ebner.
- SMITH, R. N. (1955*a*). The arrangement of the ansa spiralis of the sheep colon. *J. Anat., Lond.*, **89**, 246-249.
- SMITH, R. N. (1955*b*). Further observations on the colon primum of the sheep. *J. Anat., Lond.*, **89**, 579.

THE DEVELOPMENT AND FATE OF THE ABDOMINAL CHROMAFFIN TISSUE IN THE RABBIT

By REX E. COUPLAND

Department of Anatomy, University of Leeds

INTRODUCTION AND HISTORICAL SURVEY

Both intra- and extra-adrenal chromaffin tissue has been described in mammals for many years. In spite of this fact few accounts exist of the development, and more especially the ultimate fate, of this tissue in lower animals.

The works of Ivanoff (1925), Iwanow (1930, 1932) and Coupland (1952, 1954) on the development and fate of the chromaffin tissue have indicated that in man extra-adrenal chromaffin tissue reaches maximal development at or shortly after the time of birth, whilst the adrenal medulla is incompletely developed at this time. During early childhood the adrenal medulla quickly assumes a more fully differentiated appearance while degenerative changes are observed in the extra-adrenal chromaffin tissue in the 5-year-old child and in older specimens. In the human adult only a few small scattered masses of extra-adrenal chromaffin tissue remain in association with the pre-vertebral sympathetic plexuses. No definite evidence of adrenal medullary degeneration was reported.

The nature of the process of degeneration of extra-adrenal chromaffin tissue (para-aortic bodies) in man is not completely decided. According to Ivanoff (1925) and Iwanow (1932) a lymphocytic infiltration accompanies the change.

Coupland (1954) used fresh post-mortem material (2-12 hr. after death), obtained from cases in which the cause of death was not likely to affect either the pre-aortic region of the abdomen or, with one exception, the general lymphatic tissue. In such material there was no evidence of lymphocytic infiltration of the bodies. The degenerative changes were confined to a general increase in the supporting connective tissues of the para-aortic bodies which at the same time were becoming elongated and apparently distracted.

Although observations on a particular organ in one animal do not necessarily allow the conclusions to be applied to members of different species, the author has investigated the formation and fate of the abdominal chromaffin tissue in rodents with the hope of throwing some light upon the mechanism of post-natal changes in the chromaffin system. The use of freshly killed material allows of more accurate cytological investigation: the objections previously raised by the writer (Coupland, 1954) to detailed cytological observations on possibly abnormal tissues (Iwanow, 1932) and to material acquired many hours after death (West, Shepherd, Hunter & Macgregor, 1953) no longer obtain in such material.

Extra-adrenal chromaffin tissue has been described in the rabbit by Stilling (1890), Kohn (1903), Swale Vincent (1910), Wheeler & Swale Vincent (1917) and Wislocki (1922). Only Kohn described the pre-natal development and post-natal fate of this tissue in detail.

Kohn (1903) described the appearance of chromaffin cells in the abdominal pre-aortic region in foetuses obtained from a 15-day pregnant doe and claimed to have observed a well-defined chromaffin reaction in these cells in 21 mm. long specimens obtained from an animal which had been pregnant for 16 days. The chromaffin tissue was described as growing rapidly during foetal life and was observed in post-natal specimens aged 6 weeks, 3 months and in 'adult' animals. After birth the extra-adrenal chromaffin tissue was observed in the form of elongated strands of cells. Some of the strands were in continuity with the cells of the adrenal medulla. Degenerative changes were not reported.

MATERIALS AND METHODS

Cross-bred rabbits were used throughout. Since no sex differences were observed in the distribution or quantity of chromaffin tissue, the sex has not been stated. At least two pre-natal specimens of the following sizes were used: 4.5, 7, 8, 10, 16, 20, 30, 35, 60 and 65 mm. Two post-natal specimens of the following ages were also examined: 1,* 3, 5, 7, 12 and 14* days, 8,* 12,* 16,* 40,* and 100 weeks.

Foetal specimens were fixed in Bouin and formol-dichromate, at least one of each of the above sizes being fixed by each method. Animals were killed by a blow on the head and were fixed routinely by perfusing with formol-dichromate (5% formaldehyde, 3.5% potassium bichromate). The posterior abdominal wall was then removed and immersed in fixative for 24 hr. Some tissues were fixed in Bouin, Carnoy, formol-potassium iodate, formol-Zenker and Helly. Material was embedded in paraffin and serial sections cut at 4–8 μ . Sections were stained routinely with haematoxylin and Giemsa, alternate slides being treated by each stain. In some cases sections were stained with Masson's trichrome, Schiff's reagent, methyl green-pyronine, and Wilder's and Gomori's methods for reticular fibres. Reconstructions of the posterior abdominal walls of a number of the animals were made using the camera lucida method to indicate the disposition of the chromaffin tissue.

4.5 mm.

Pre-natal development

A simple neural tube exists with ependymal, mantle and marginal zones and a large central canal. Ventral and dorsal nerve roots unite to form spinal nerves which run ventrally around the body wall and towards the limb-buds. Rami communicantes are not observed, and there is no evidence of either the primitive sympathetic chain or mesodermal portions of the adrenal glands. In the thoracic region an occasional cell lies in the junctional zone between the ventral nerve root and the ventro-lateral aspect of the neural tube and appears to be crossing the external limiting membrane.

7–10 mm.

Primitive sympathetic cells, distinguishable by their marked nuclear and cytoplasmic basophilia, form a chain along the dorso-lateral aspect of the dorsal aorta. These cells contain single rounded or ovoid nuclei 6–7 μ in diameter. Mitotic figures are numerous. Rami communicantes may be traced from the spinal nerves to the

* One of each of these specimens was a litter-mate.

primitive sympathetic chain in all embryos. Longitudinal fibres have not been observed in the primitive sympathetic chain at this stage. Cells may be traced along spinal nerves from both dorsal and ventral nerve roots. As in the previous specimen, an occasional cell appears to be crossing the external limiting membrane of the thoracic portion of the spinal cord and entering the ventral nerve root (Pl. 1, fig. 1). At 7 mm. the rudiment of the mesodermal portion of the adrenal gland is visible approximately 0.5 mm. rostral to the origin of the superior mesenteric artery. This anlage lies in the angle between aorta, mesentery and developing kidney, and is intimately associated with cuboidal cells which line the coelom at this point (Pl. 1, fig. 2). Many mitotic figures are observed in these cells. Primitive sympathetic cells may be traced around the sides of the abdominal aorta and into the pre-aortic region (Pl. 1, fig. 3). They are related to the medial aspect of the mesodermal adrenal; at 7 mm. this relationship is not intimate, but by 10 mm. the two tissues are juxtaposed.

16 mm.

Primitive sympathetic cells form a para-vertebral chain in which longitudinal fibres are now visible. Large collections of these cells also lie ventral to the abdominal aorta in the region caudal to the mesodermal adrenal glands.

The mesodermal adrenal is now relatively large and is made up of rounded cells with moderately basophilic nuclei 7–8 μ in diameter and prominent nucleoli; the cytoplasm forms a uniform peripheral rim. Medially the mesodermal adrenal is intimately related to groups of primitive sympathetic cells, but in some parts the two are separated by a different type of cell (Pl. 1, fig. 4). These interposed cells are elongated with ovoid moderately or slightly basophilic nuclei 9–12 μ in diameter; each nucleus contains a prominent nucleolus and other discrete granules of chromatin. The cytoplasm is moderately abundant, but does not give a positive chromaffin reaction.

Similar cells are observed in the pre-aortic region caudal to the adrenal glands and may be arranged in whorls (Pl. 1, figs. 5–7). These cells are intimately associated with, and apparently derived from, the sympathetic elements and are considered to be phaeochromoblasts. Mitotic figures are frequently observed in both primitive sympathetic cells and phaeochromoblasts.

20 mm.

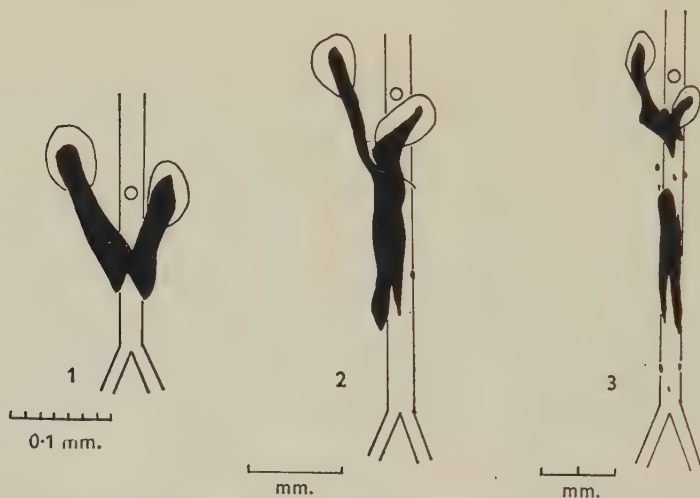
The phaeochromoblasts form a large discrete mass in the pre-aortic region (Text-fig. 1). Rostrally these cells form two discrete prolongations which are capped by the mesodermal adrenals. A section through the developing adrenal at this stage shows a well-defined peripheral cortex of mesodermal origin and a medulla (Pl. 1, fig. 8), which is composed largely of phaeochromoblasts. Associated with the phaeochromoblasts are a few cells which have faintly basophilic rounded or ovoid nuclei 7–10 μ in diameter, each nucleus containing a small nucleolus and few granules of chromatin. These cells do not give a positive chromaffin reaction, but are identical in all other respects with the phaeochromocytes of later stages and are considered to be phaeochromocytes. A few primitive sympathetic cells are associated with the phaeochromoblasts and phaeochromocytes.

30 and 35 mm.

A Y-shaped mass of chromaffin tissue lies in the pre-aortic region (Text-fig. 2), the upper extremities being capped by the mesodermal adrenal glands. The majority of the cells in this mass give a positive chromaffin reaction and conform in structure to the phaeochromocytes of older specimens. A few phaeochromoblasts and primitive sympathetic cells are also observed. Mitotic figures are often observed in this tissue.

60 and 65 mm.

The general distribution of the chromaffin tissue (Text-fig. 3) is similar to that in earlier specimens. In this specimen the main para-aortic body is, however, interrupted caudal to the adrenal glands. Small collections of chromaffin cells are occasionally



Text-fig. 1. Reconstruction of the abdominal phaeochromoblasts of a 20 mm. rabbit embryo. The origin of superior mesenteric artery is indicated.

Text-fig. 2. Reconstruction of the abdominal chromaffin tissue of a 35 mm. rabbit foetus. The origin of superior mesenteric artery is indicated.

Text-fig. 3. Reconstruction of the abdominal chromaffin tissue of a 60 mm. rabbit foetus. The origin of superior mesenteric artery is indicated.

observed in the lumbar paravertebral ganglia, but have not been observed in the thoracic region. Small groups of chromaffin cells are also observed in the pre-aortic plexus and these are indicated in Text-fig. 3. The chromaffin cells in all areas appear identical in structure after fixation in formol-dichromate, formol-iodate and Bouin and staining with Ehrlich's haematoxylin, iron-haematoxylin or Giemsa.

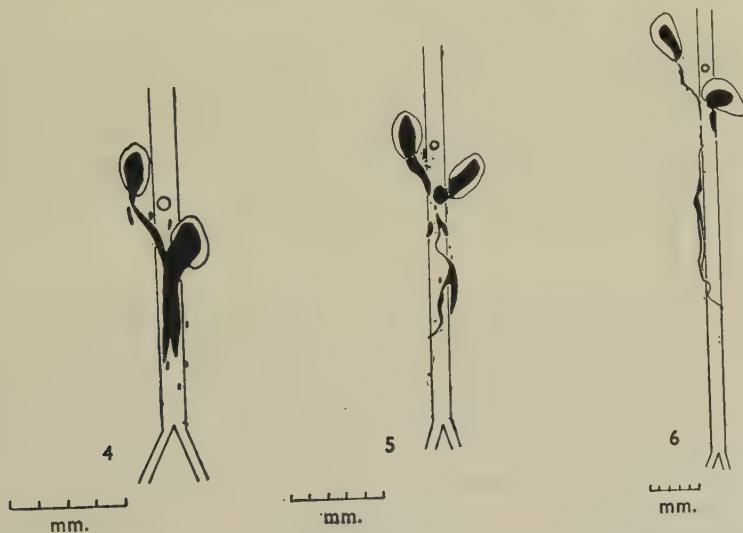
The chromaffin cells of the para-aortic bodies form cords in close association with capillary blood vessels (Pl. 1, fig. 9). Those of the adrenal medulla are more obviously related to the tributaries of the adrenal vein. Supporting connective tissue is only visible after silver impregnation and is reticular in type. Mitotic figures are numerous in both intra- and extra-adrenal chromaffin tissue.

1-14 days

Post-natal

Intra- and extra-adrenal chromaffin tissue is continuous in all specimens (Text-figs. 4, 5). Sections through the main para-aortic bodies (Pl. 2, figs. 1, 2) show discrete masses of chromaffin tissue which are surrounded by a thin connective tissue capsule. Chromaffin cells are arranged in cords in association with capillary blood vessels. Supporting stroma is reticular in type in all except periarterial areas, where a small amount of collagenous tissue may be observed.

The extra-adrenal chromaffin tissue of the 7-day-old specimen (Text-fig. 5) is becoming elongated and narrowed in comparison with earlier specimens. Small discrete collections of chromaffin cells are scattered throughout the pre-aortic sym-



Text-figs. 4-6. Reconstruction of the abdominal chromaffin tissue of rabbits aged 1 day, 7 days and 8 weeks respectively. The origins of superior mesenteric arteries are indicated.

pathetic plexus, and are occasionally observed in the lumbar para-vertebral ganglia. The main para-aortic body extends caudally, passing posterior to the left renal vein. No definite difference has been observed between the intra- and extra-adrenal chromaffin cells after using a variety of methods of fixation and staining. Cells in all situations give a positive chromaffin reaction and show faint brown granules after formol-iodate fixation; the nucleus is round or ovoid, 7-10 μ in diameter, and contains a small nucleolus and a few scattered chromatin granules (Pl. 2, fig. 3). Mitotic figures are observed.

In the adrenal medulla the chromaffin cells are arranged in columnar fashion around the tributaries of the adrenal vein. Cords of cells from zona reticularis penetrate the medulla.

Primitive sympathetic cells and phaeochromoblasts have not been observed in these specimens.

8 weeks

The distribution of chromaffin tissue is indicated in Text-fig. 6. A long thin para-aortic body lies in the pre-aortic region, bifurcating caudally. There has been a marked increase in length of the main para-aortic body with an associated slight reduction in cross-sectional area (Pl. 2, fig. 4). Small groups of chromaffin cells may be observed in the majority of the pre- and para-vertebral sympathetic ganglia. Intra- and extra-adrenal chromaffin tissue is again continuous.

No mitotic figures have been observed in 100 sections through the main para-aortic body, and only one mitotic figure has been observed in 100 sections of the adrenal medullae. No degenerative changes are observed in chromaffin tissue. The chromaffin reaction is intense in both adrenal medullae and para-aortic bodies.

12 weeks

The general distribution of chromaffin tissue is similar to that of the last specimen. No mitotic figures were observed in either the adrenal medullae or the extra-adrenal chromaffin tissue. Continuity between intra- and extra-adrenal tissue is observed on both sides. Chromaffin cells have the same morphological characters as in previous specimens (Pl. 2, fig. 6). A small amount of collagenous supporting stroma may be observed in some sections through the para-aortic bodies.

16 weeks

Chromaffin tissues exists as elongated strands in the pre-aortic region (Text-fig. 7) and extends from the right adrenal gland to a point 4.5 cm. caudal to this structure. It is, therefore, almost twice the length of the extra-adrenal chromaffin tissue of the 8-week-old specimen. In many places the strands are only a few cells thick. The chromaffin reaction is intense in both adrenal glands and para-aortic bodies. Mitotic figures have not been observed in chromaffin tissue. There is no evidence of cellular degeneration or infiltration, but the para-aortic bodies now show a definite connective stroma after routine stains, the connective tissue being most marked in the vicinity of small arteries.

Longitudinal sections through the main para-aortic body show chromaffin cells lying with the long axes of their nuclei parallel to the long axis of the body (Pl. 2, fig. 5).

40 weeks

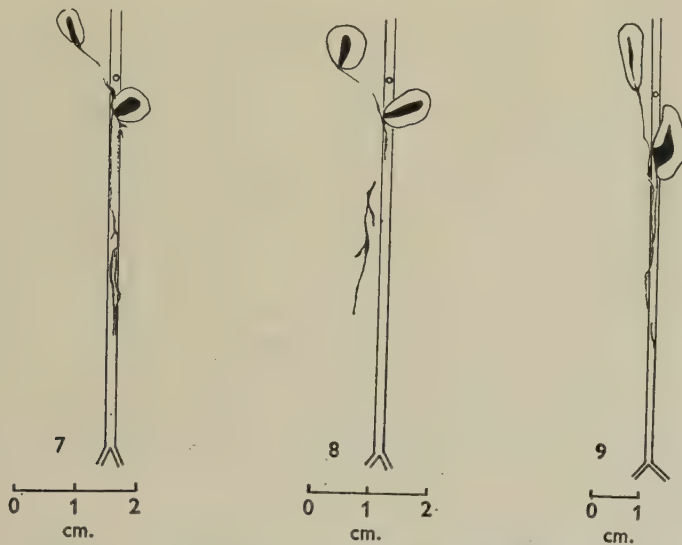
The general distribution of tissue is similar to that in the previous specimen. The main para-aortic body, indicated in Text-fig. 8, has been displaced laterally during the embedding process, but is still intimately associated with the pre-aortic sympathetic plexus. There is a marked increase in the supporting connective tissue in the para-aortic bodies (Pl. 2, figs. 7, 8), and the chromaffin cells at times appear to be completely surrounded by collagenous fibres.

There is no evidence of cellular infiltration of the bodies. An occasional pyknotic nucleus is observed in both the para-aortic bodies and in the adrenal medullae but these are few and there is no evidence of active cellular degeneration in any area. The chromaffin reaction is strongly positive in all areas.

The thoracic sympathetic chains of two 40-week-old animals were examined for chromaffin cells. None were observed. A few small groups of chromaffin cells were observed in the lumbar paravertebral ganglia of both animals.

100 weeks old

Extra-adrenal chromaffin tissue exists in the form of thin attenuated strands which extend from the right adrenal gland to a point 5.5 cm. caudal to this structure. Cells are sparsely distributed amongst abundant supporting connective tissue (Pl. 2, fig. 9), and although a positive chromaffin reaction occurs this is sometimes



Text-figs. 7-9. Reconstruction of the abdominal chromaffin tissue of rabbits aged 16, 40 and 100 weeks respectively. The origins of superior mesenteric arteries are indicated.

less intense in some of the extra-adrenal cells than in the medullary phaeochromocytes. An attenuated strand of chromaffin cells runs from each adrenal medulla to the pre-aortic region.

There is no evidence of cellular infiltration. Occasional pyknotic nuclei are seen in both adrenal medullae and para-aortic bodies, but there is no evidence of widespread degeneration in any area.

The number of extra-adrenal phaeochromocytes appears to be definitely reduced as compared with those of the 4-month-old specimens.

DISCUSSION

Kohn (1903) observed the first signs of differentiation of chromaffin tissue from the sympathetic anlage in a 15-day-old rabbit foetus. In the present work similar observations have been made in 16 mm. foetuses (14 days). In Kohn's description no mention was made of an intermediate cell between the primitive sympathetic elements and the fully developed phaeochromocyte. The present writer has observed an intermediate form, the phaeochromoblast, in rabbit foetuses of 16 and 20 mm.;

this is larger than either the primitive cell or the fully differentiated chromaffin cell, and does not give a positive chromaffin reaction. Similar cells were observed in the 14–19.5 mm. human foetuses (Coupland, 1952).

In the foetal rabbit, as in foetal man, there is a greater amount of extra-adrenal than of intra-adrenal chromaffin tissue. During early post-natal life there is a rapid increase in the amount of the intra-adrenal component, and mitotic figures have been observed in the adrenal medulla in an 8-week-old animal but not in older specimens. This finding is in keeping with the work of Elliott & Tuckett (1906), who found little increase in the size of the rabbit adrenal medulla after the animals had reached a weight of 900 g.

There is a marked elongation of the main para-aortic bodies during the first few weeks of post-natal life. The main mass of tissue extends along the pre-aortic region for a distance of 6 mm. at birth, 10 mm. at 7 days, 25 mm. at 8 weeks, 50 mm. at 16 weeks, 40 mm. at 40 weeks, 50 mm. at 100 weeks. This elongation can be correlated with the length of abdominal aorta, and is accompanied by a reduction in the cross-sectional area of the chromaffin tissue of the main body. This gradual decrease in the amount of chromaffin tissue per section cannot be accurately represented on the line drawings because of the scale employed, and because of a concomitant increase in the amount of supporting tissue; the change may, however, be followed by comparing Text-figs. 4–9 and Pl. 2, figs. 1, 2, 4 and 7.

Mitotic figures were not observed in the para-aortic bodies of 8-week-old animals or in older specimens. The elongation of the main para-aortic bodies is accompanied by an alignment of the nuclei of the chromaffin cells with their long axes parallel to the length of the aorta. This change would be in keeping with a process of distraction due to relative growth rates as was postulated in the 5- to 7-year-old human foetus (Coupland, 1954).

An increase in the connective tissue stroma of the para-aortic bodies with the appearance of collagenous fibres has been observed in animals aged 7 days and over, and becomes a marked feature after 12 weeks. Similar changes occur in the child between the ages of 3–5 years (Coupland, 1954). A slight increase in the supporting tissue of the adrenal medulla also occurs but this is always very much less evident than in the para-aortic bodies, and even in animals aged 100 weeks the collagenous fibres are few in number and the stroma is still mainly reticular. The significance of the increase in stroma is difficult to assess. Bauchmann (1937), Dribben & Wolfe (1947) and Meyers & Charipper (1956) considered that the functional activity of cells could be correlated with the amount of supporting stroma, and found that in the adrenal cortex stroma increased with age, the increase being most apparent in the zona reticularis. Applying this hypothesis to the chromaffin tissue the findings would indicate that the extra-adrenal chromaffin tissue is less active than the adrenal medulla.

On the other hand, the more abundant stroma in the para-aortic bodies may be correlated with their relatively unsupported and unprotected position, as compared to the adrenal medulla. This would become more apparent with age, as a general increase in the stroma of the various organs appears to be a natural accompaniment of the ageing phenomenon. In some organs this change begins a few days after birth (Wolfe, Burack, Lansing & Wright, 1942).

Cellular infiltration of chromaffin tissue has not been observed in any animals, and there is no evidence to suggest that a cellular infiltration is in any way connected with the age changes observed in the para-aortic bodies of the rabbit.

Yeakel (1947) observed adrenal medullary hyperplasia in Wistar rats which were more than 700 days old. No such change has been observed in rabbits of the same age.

A well-developed chromaffin reaction was observed in both intra- and extra-adrenal chromaffin tissue in all post-natal specimens. This finding suggests that the extra-adrenal chromaffin tissue of the rabbit may be functionally active throughout life. Caution must, however, be exercised in making such an assumption without experimental evidence, since a positive chromaffin reaction has been observed in adrenal medullary autografts for periods up to 6 months after implantation (Coupland, 1955).

Small groups of chromaffin cells have been observed in the lumbar para-vertebral sympathetic ganglia in both pre- and post-natal life. They appear identical in structure with those elsewhere. These elements have not been observed in the thoracic ganglia in the present work.

During the present investigation large groups of cells were seen migrating along the spinal nerves, from the region of the dorsal root ganglion, in 4.5, 7 and 8 mm. embryos. A few cells were also observed in association with the ventral nerve roots, and in a small number of sections through the thoracic region at this stage (4.5–8 mm.) cells were observed apparently crossing the external limiting membrane and entering the ventral nerve root (Pl. 1, fig. 1). These cells are similar structurally to the cells which can be traced along the spinal nerves to the sympathetic anlage and the finding would tend to support the views of Kuntz (1926) and Jones (1939, 1941) that the primitive sympathetic elements originate in part from the neural tube.

SUMMARY

1. Phaeochromoblasts appear in the pre-aortic region of the 16 mm. rabbit foetus.
2. A positive chromaffin reaction is observed in the chromaffin tissue of the 30 mm. rabbit foetus and in all older specimens.
3. Intra- and extra-adrenal chromaffin tissue is continuous in both pre- and post-natal life, although the association is less obvious in the adult animal.
4. No evidence of growth of chromaffin tissue has been observed in animals aged more than 8 weeks.
5. There is a post-natal elongation of the main para-aortic bodies which is later associated with a marked increase in connective tissue stroma.
6. There is no evidence of cellular infiltration of chromaffin tissue in the rabbit.

I wish to thank Prof. A. Durward for the facilities placed at my disposal and for reading this manuscript, Dr W. K. J. Walls for the photographic work and Mr R. K. Adkin for assistance with the histological material.

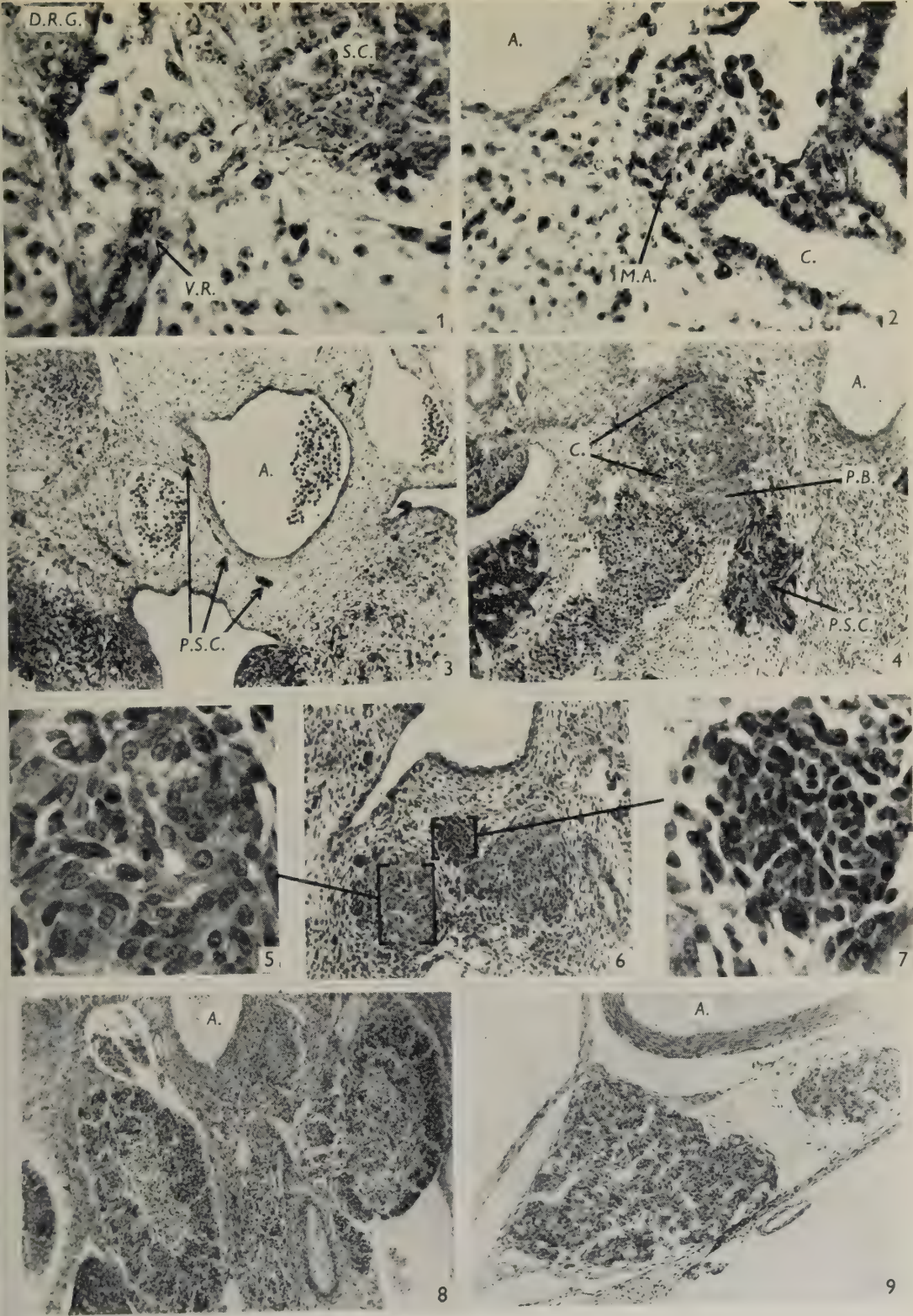
REFERENCES

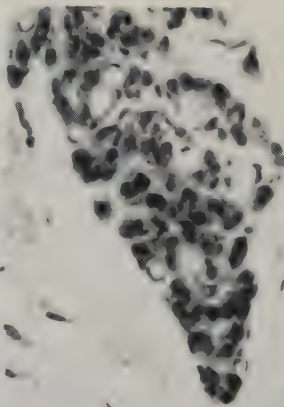
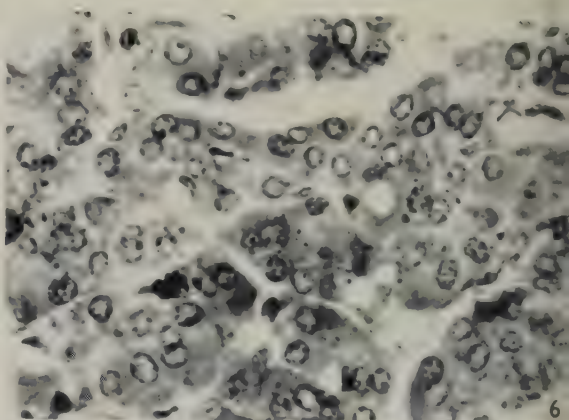
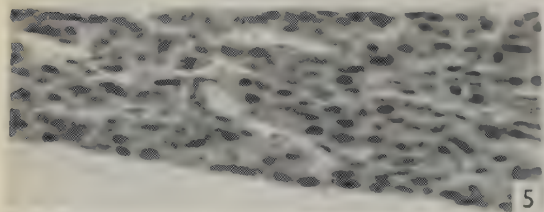
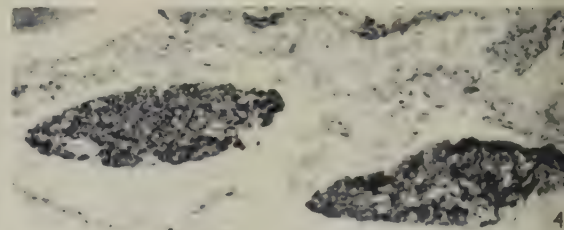
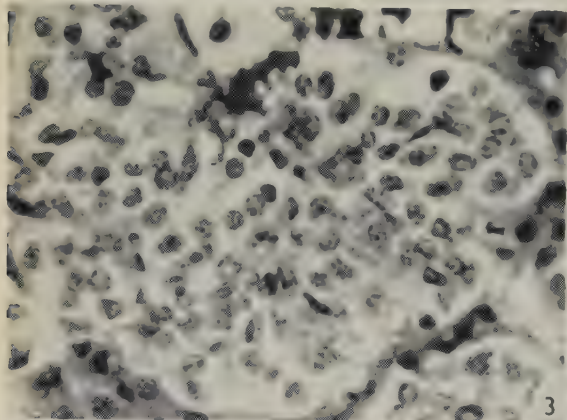
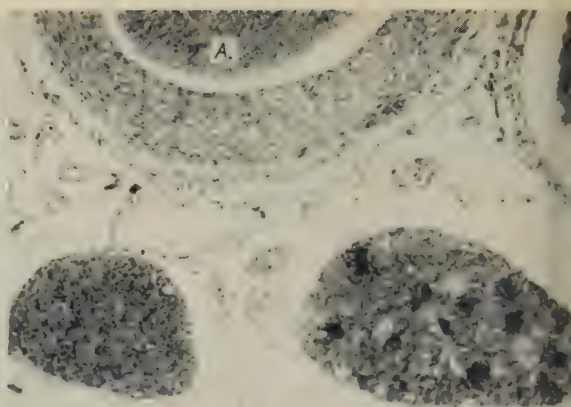
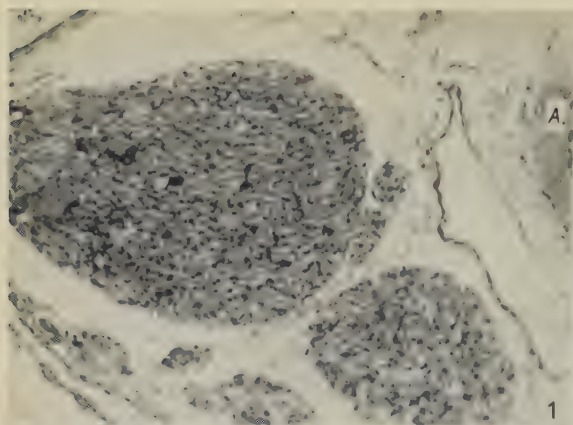
- BAUCHMANN, R. (1937). Über die Bedeutung des argyrophilen Bindgewebes (Gitterfasern) in der Nebennierenrinde und im Corpus Luteum. *Z. Mikr.-anat. Forsch.* **41**, 433-446.
- COUPLAND, R. E. (1952). The prenatal development of the abdominal para-aortic bodies in man. *J. Anat., Lond.*, **86**, 357-372.
- COUPLAND, R. E. (1954). Post-natal fate of the abdominal para-aortic bodies in man. *J. Anat., Lond.*, **88**, 455-464.
- COUPLAND, R. E. (1955). Cortical zonation and survival of chromaffin cells in adrenal autografts. *Nature*, **175**, 211-212.
- DRIBBEN, I. S. & WOLFE, J. M. (1947). Structural changes in connective tissue of the adrenal glands of female rats associated with advancing age. *Anat. Rec.* **98**, 557-585.
- ELLIOTT, T. R. & TUCKETT, I. (1906). Cortex and medulla in the suprarenal glands. *J. Physiol.* **34**, 332-369.
- IVANOFF, G. F. (1925). Zur Frage über die Genese und Reduktion der Paraganglien des Menschen. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **77**, 234-244.
- IWANOW, G. (1930). Variabilitäten der abdominalen Paraganglien in Kindesalter. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **91**, 405-441.
- IWANOW, G. (1932). Das chromaffine und interrenale System des Menschen. *Z. ges. Anat. 3. Ergebn. Anat. EntwGesch.* **29**, 87-280.
- JONES, D. S. (1939). Studies on the origin of sheath cells and sympathetic ganglia in the chick. *Anat. Rec.* **73**, 343-358.
- JONES, D. S. (1941). Further studies on the origin of the sympathetic ganglia in the chick embryo. *Anat. Rec.* **79**, 7-15.
- KOHN, A. (1903). Die Paraganglien. *Arch. mikr. Anat.* **62**, 263-365.
- KUNTZ, A. (1926). The role of cells of medullary origin in the development of the sympathetic trunks. *J. Comp. Neurol.* **40**, 389-408.
- MEYERS, M. W. & CHARIPPER, H. A. (1956). A histological and cytological study of the adrenal gland of the golden hamster (*Cricetus auratus*) in relation to age. *Anat. Rec.* **124**, 1-25.
- STILLING, H. (1890). A propos de quelques expériences nouvelles sur la maladie d'Addison. *Rev. Médecine*, **10**, 808-831.
- VINCENT, SWALE (1910). The chromaffin tissues and the adrenal medulla. *Proc. Roy. Soc. B*, **82**, 502-515.
- WEST, G. B., SHEPHERD, D. M., HUNTER, R. B. & MACGREGOR, A. R. (1953). The function of the organs of Zuckerkandl. *Clin. Sci.* **12**, 317-326.
- WHEELER, T. D. & VINCENT, SWALE (1917). The question as to the relative importance to life of cortex and medulla of the adrenal bodies. *Trans. Roy. Soc., Can.*, **11**, 125-127.
- WISLOCKI, G. B. (1922). Note on a modification of the chromaffin reaction, with observations on the occurrence of abdominal chromaffin bodies in mammals. *Johns Hopk. Hosp. Bull.* **33**, 359-361.
- WOLFE, J. M., BURACK, E., LANSING, W. & WRIGHT, A. W. (1942). The effects of advancing age on the connective tissue of the uterus, cervix and vagina of the rat. *Amer. J. Anat.* **70**, 135-165.
- YEAKEL, E. H. (1947). Medullary hyperplasia of adrenal glands in aged Wistar and grey Norway rats. *Arch. Path. (Lab. Med.)*, **44**, 71-77.

EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Transverse section through the thoracic region of a 7 mm. rabbit embryo. *D.R.G.*, dorsal root ganglion; *V.R.*, ventral nerve root; *S.C.*, ventral aspect of spinal cord. Bouin. Iron haematoxylin. $\times 342$.
- Fig. 2. Transverse section through the developing mesodermal adrenal of a 7 mm. rabbit embryo. *A.*, aorta; *M.A.*, adrenal; *C.*, coelom. Bouin. Iron haematoxylin, $\times 342$.
- Fig. 3. Transverse section through the abdomen of a 7 mm. rabbit embryo. Primitive sympathetic cells (*P.S.C.*) are extending into the pre-aortic region. *A.*, aorta. Bouin. Haematoxylin and eosin. $\times 76$.





- Fig. 4. Transverse section through the upper abdomen of a 16 mm. rabbit foetus. *A.*, aorta; *P.S.C.*, primitive sympathetic cells; *P.B.*, phaeochromoblasts; *C.*, primitive adrenal cortical cells. Bouin. Haematoxylin and eosin. $\times 104.5$.
- Fig. 5. Phaeochromoblasts of a 16 mm. rabbit embryo. Enlargement of cells shown in fig. 6. Bouin. Haematoxylin and eosin. $\times 342$.
- Fig. 6. Transverse section through the mid-abdomen of a 16 mm. rabbit embryo. Collections of intensely basophilic primitive sympathetic cells lie in the midline. On each side are whorls of phaeochromoblasts.
- Fig. 7. An enlargement of the primitive sympathetic cells of fig. 6. Bouin. Haematoxylin and eosin. $\times 342$.
- Fig. 8. Transverse section through the developing adrenals of a 20 mm. rabbit embryo showing a peripheral adrenal cortex and a central medulla. *A.*, aorta. Formol-dichromate. Haematoxylin and eosin. $\times 76$.
- Fig. 9. Section through a para-aortic body of a 60 mm. rabbit foetus. Cords of phaeochromocytes are associated with capillary blood-vessels. *A.*, aorta. Formol-dichromate. Ehrlich's haematoxylin. $\times 104.5$.

PLATE 2

- Fig. 1. Transverse section through the main para-aortic bodies of a 3-day-old rabbit. *A.*, aorta. Bouin. Masson's trichrome. $\times 110$.
- Fig. 2. Transverse section through the main para-aortic bodies of a 7-day-old rabbit. *A.*, aorta. Formol-dichromate. Haematoxylin and eosin. $\times 80$.
- Fig. 3. Phaeochromocytes of the adrenal medulla of a 12-day-old rabbit. Carnoy. Iron haematoxylin. $\times 490$.
- Fig. 4. Transverse section through the main para-aortic bodies of an 8-week-old rabbit. Formol-dichromate. Haematoxylin and eosin. $\times 80$.
- Fig. 5. Longitudinal section through an elongated para-aortic body of a 16-week-old rabbit. Nuclei arranged with their long axes parallel to the length of the body. Formol-dichromate. Ehrlich's haematoxylin. $\times 360$.
- Fig. 6. Transverse section through the main para-aortic body of a 12-week-old rabbit. Formol-Zenker. Iron haematoxylin. $\times 490$.
- Fig. 7. Transverse section through the main para-aortic body (*P.A.B.*) of a 40-week-old rabbit. Formol-dichromate. Ehrlich's haematoxylin. $\times 80$.
- Fig. 8. Enlargement of part of the para-aortic body shown in fig. 7. Formol-dichromate. Ehrlich's haematoxylin. $\times 360$.
- Fig. 9. Section through the main para-aortic body of a 100-week-old animal. Formol-dichromate. Ehrlich's haematoxylin. $\times 490$.

THE ROLE OF THE PERITONEUM IN THE FORMATION OF THE SEPTUM RECTO-VESICALE

By P. H. S. SILVER

Department of Anatomy, Middlesex Hospital Medical School, London, W. 1

‘In the fourth-month (human) foetus the recto-vesical pouch is relatively very much deeper than in the adult, and the subsequent alteration in its depth is brought about by the obliteration of the lower portion of the pouch by the fusion of its walls. This occluded portion persists as a strong sheet, termed the recto-vesical septum (septum of Denonvilliers). . . .’

This quotation from Buchanan (1949) describes a widely held view (Cunningham, 1951; Gray, 1954) concerning the origin of the septum recto-vesicale. A survey of the literature, however, has revealed remarkably little evidence in support of such an account, and indeed, of what there is, much does not stand up to critical examination.

With regard to the role of the peritoneum in the formation of the septum, there are no less than three radically different accounts in the literature:

(1) Peritoneal fusion occurs and gives rise to the septum (Cuneo & Veau, 1899; Elliot Smith, 1908; Tobin & Benjamin, 1945; Uhlenhuth, Wolfe, Smith & Middleton, 1948).

(2) Fusion occurs but does not give rise to the septum which is formed later by the condensation of loose areolar tissue (Wesson, 1922, 1923).

(3) Fusion does not occur (Borghese, 1937).

It is necessary to examine these accounts in detail.

Cuneo & Veau (1899) first enunciated the theory of peritoneal fusion with the simultaneous formation of the septum recto-vesicale. Elliot Smith (1908) supported their interpretation; but it is noteworthy that he offered no histological evidence of fusion, nor was fusion actually stated to have been observed. He was influenced by the relationship of the pouch anteriorly to the prostate during the fourth month of development, and argued that, since in the definitive position the pouch is related not to the prostate but to the bladder, upward migration would have to take place during later development. Such an argument, however, cannot carry conviction unless it is first shown that the bladder and prostate do not migrate downwards from their earlier position. The possibility that descent of the bladder might occur in the foetus and play a part in the change of relationship between the pouch and bladder has never been discussed. This omission in the literature is surprising, for it is well known that the bladder descends during infancy, and there is no *a priori* reason why this descent should not begin before birth.

Now if upward migration of the pouch does occur during development, it would be expected that not only would there be a change in its relationship to the prostate and bladder in front, but also that it would draw nearer to the sacral promontory above, draw away from the surface of the perineum below, and ascend in relation to the rectum behind. No confirmatory evidence of this kind was produced by Elliot

Smith. So far as the rectum is concerned, figures quoted by Silver (1955) show the pouch to be situated from the fourth month onwards, either just above or just below the main plica transversalis recti (Houston's main valve), i.e. in the *definitive* position. Any account of the fate of the pouch in the foetus must explain how, from the fourth month onwards, the anterior relations can change while the posterior relations remain constant. Even if Elliot Smith had actually demonstrated upward migration, he had still to establish that this was brought about by peritoneal fusion, and further, once this had been done, he had lastly to show the developmental relationship between fusion and septum formation.

Wesson (1922, 1923) first claimed to have shown histological evidence of peritoneal fusion, but he stated categorically that the septum was not formed in this way, but by the condensation of loose areolar tissue. Tobin & Benjamin (1945) made a similar claim regarding fusion, but concluded that the septum was derived from the peritoneum. However, the histological evidence of peritoneal fusion in these two papers is not convincing. In only a single embryo did Wesson (1922) find a raphé (see his fig. 3, 25 mm. embryo), and this he identified as having been produced by peritoneal fusion; but the section is poorly fixed, as evidenced by the shrunken rectal mucosa. Similar appearances have been seen in the present study, but always in imperfectly fixed material. No such raphé was described by Tobin & Benjamin (1945). Both papers agree, however, in describing 'cysts' just below the bottom of the pouch, marking the places where fusion is as yet incomplete. But it is difficult for the reader to assess the nature of the structures identified as 'cysts' owing to the low magnification of the figures (see Wesson's fig. 4, 'Carnegie Inst. Embr. 404, slide 12, row 2, sect. 2'; and Tobin & Benjamin's fig. 5, 'Carnegie Inst. Embr. 405, slide 12, row 2, sect. 2'). Although the reference numbers of these embryos are different, a study of the fine detail suggested that they are two photographs of the self same section. Ebert (1956) has confirmed that this is, in fact, the case. The evidence regarding 'cysts' therefore relies on the appearance shown in a single embryo. Cysts have not been seen in the present study. Uhlenhuth *et al.* (1948) examined foetuses aged from 6 months upwards, but did not examine the pouch histologically.

At variance with all these accounts is that of Borghese (1937), who after a comprehensive study of embryos and foetuses, found no evidence of peritoneal fusion. The change in the anterior relations he attributed to a process of 'unfolding' which gradually reduced the depth of the recto-vesical pouch. If his account is correct, then the septum recto-vesicale must be formed independently of the peritoneum.

Finally, mention must be made of the muscle fibres whose presence in the septum was first described by Denonvilliers (1836). Cuneo & Veau (1899) admitted that the presence of these fibres did not fit well with the suggested peritoneal origin, but added that they had failed to demonstrate them in their embryos. Tobin & Benjamin (1945) stated that 'the embryological origin... of the smooth muscle in this membrane is not clear'. In the present communication their existence in the foetus will be demonstrated: the present study has shown that in fact they are continuous with the external longitudinal coat of the rectum.

MATERIAL AND METHODS

The present investigation has necessarily been concentrated upon the male, but data relating to the female are included, the term recto-vaginal septum being then used in the text.

The material examined, fifty-two embryos and foetuses, is shown in Table 1. Embryos of less than 30 mm. crown rump length were fixed by immersion, otherwise fixation was always carried out by intravascular injection. The practice of opening the abdominal cavity of larger foetuses in order to help fixation produces considerable distortion, particularly of the bladder, and in consequence was never followed. In certain foetuses examined, pressure on the anterior abdominal wall had forced abdominal viscera into the pelvis, whereby the recto-vesical pouch had become distended and deepened with resultant alteration of its relations. None of these specimens is included among the present data.

Specimens for histological examination were embedded in wax and celloidin. Those blocks which were to be cut sagittally were mounted with the vertebral column parallel with the edge of the knife, so that any distortion produced would be from before backwards and not from below upwards. This is an important point when upward movement of the pouch is being studied. Certain specimens were examined by naked eye after hand section of the pelvis in the sagittal plane; tissue from the bottom of the pouch was also taken for histological examination.

The following points were investigated:

(1) Histological evidence of peritoneal fusion in the recto-vesical pouch. The appearances observed were compared with those of the peritoneal membrane in regions where fusion is known to occur.

(2) The immediate anterior and posterior relations of the pouch. In Table 1 the anterior relationships are recorded in three positions: (1) below the opening of the common ejaculatory ducts into the urethra; (2) between these openings and the bladder neck; and (3) above the bladder neck. Behind, the position of the pouch was recorded relative to the position of Houston's main valve. This was done by measuring the distances of the valve and of the pouch from the sacral promontory, and recording the valve as + (i.e. above the pouch) and - (i.e. below).

(3) Evidence of upward migration was also searched for by measuring the distance from the sacral promontory to the pouch, and also from the promontory to the surface of the perineum immediately in front of the anus. The former measurement was then expressed as a percentage ratio of the latter. The rationale of the method is as follows: as upward migration occurs, the distance promontory-to-pouch will form a smaller and smaller ratio of the distance promontory-to-perineum. Reference to Pl. 1, fig. 4, will make this easier to follow. It will also be obvious from the figure that: (1) a similar change in the ratio could be produced by a downward movement or rotation of the perineum; such movements occur during the development of the structures in the deep and superficial pouches; and (2) that if the pouch is to maintain a constant relationship to both the promontory and the perineum during downward movement of the perineum, then the pouch would also migrate *downwards*. A fall in the ratio indicates therefore either upward migration of the pouch or downward movement of the perineum, but does not distinguish between them.

(4) The position of the bladder. No convenient bony landmark was found by reference to which the position of the bladder (e.g. the internal meatus) could be located. Use was made, however, of the direction of the true ligaments of the bladder. These ligaments are attached at the junction of prostate and bladder neck. In the foetus they pass vertically upwards from their attachment to the pelvic wall: in the definitive position they lie horizontally. Since their visceral and pelvic attachments do not change, the direction of these ligaments will therefore act as a guide to the position of the bladder neck, passing vertically upwards when the bladder neck is high up, and passing horizontally when the bladder neck has reached its definitive position low down in the pelvis. In examining the position of the bladder in whole sections of the pelvis (Pl. 1, figs. 3, 7) a seeker was always introduced into the ureteric orifice. The foetal bladder has an extremely narrow neck, and in certain specimens the ridge of the interureteric bar can easily be mistaken for the internal meatus. The identification of the ureteric opening ensures against this mistake.

(5) The time of appearance and histological features of the septum recto-vesicale.

RESULTS

(1) *Histological evidence of peritoneal fusion.*

Pl. 1, fig. 1, shows the histological appearances of true peritoneal fusion or zygosis (Keith, 1914, 1946) taking place between the posterior abdominal wall and the posterior surface of the duodenal mesentery in a 39 mm. embryo. There is considerable hyperplasia of the peritoneal lining in this region, and, in addition, cell islands and isolated cells can be seen which have lost their original continuity with the peritoneum and are sinking into the loose extraperitoneal connective tissue. In older embryos these isolated cells are replaced by a well-marked sheet of connective tissue. Appearances such as this have never been seen at the bottom of the recto-vesical pouch.

There is no doubt that true fusion is an active developmental process and does not result merely from the juxtaposition and adhesion of two layers of peritoneum. It is not possible to prove the negative statement that fusion does not occur in a given site, but if it is logical to expect fusion in one part of the peritoneal cavity to resemble fusion in another part of the same cavity, then the negative findings reported above are important. These findings are in agreement with those of Borghese (1937).

Two artefacts may be mentioned at this point. First, in so-called cross-sections of whole embryos, owing to the extreme flexion of the tail region, the knife cuts through the pelvis at an increasingly oblique angle, so that the tip and lateral recesses of the pouch are cut tangentially, and here the peritoneum appears thickened due to the plane of section. This thickening may be mistaken for fusion. The second source of error, illustrated in Pl. 1, fig. 2, is produced by block compression and results in the anterior and posterior layers of the pouch being pinched together.

(2) *Relations of the recto-vesical pouch to the genito-urinary system and rectum*

Table 1 shows the relations of the pouch anteriorly to the prostate and bladder. Up to about the middle of the fourth month (14-15 weeks) the pouch lies below the

Table 1

Crown rump length (mm.) (1)	Plane of section (2)	Promontory- to-pouch (A) (3)	Promontory- to-perineum (B) (4)	A/B × 100 (5)	Anterior relation of recto- vesical pouch (6)	Promontory to Houston's main valve (7)
9	S	0.7	0.9	77	1	—
11.5	S	0.65	1.1	54	1	—
13.5	S	0.7	1.41	49.6	Female	—
13.5	S	1.1	1.4	78.5	1	—
14	S	1.15	1.65	69.7	1	—
15	S	1	1.15	86.9	1	—
16	T	—	—	—	—	—
17	T	—	—	—	—	—
19.5	S	1.95	3.15	62	1	—
21	S	1.1	2.1	52.4	1	—
21.5	S	1.35	2.25	59.9	1	—
23.5	T	—	—	—	—	—
25	S	2	3.75	53.4	1	—
27	T	—	—	—	Female	—
27	T	—	—	—	—	—
29	S	1.75	3.1	56.4	1	—
33.5	T	—	—	—	—	—
35	S	2.3	4.0	57.4	1	—
37	T	—	—	—	1	—
39	S	2.8	4.5	62.2	Female	—
42	S	1.75	3.4	51.4	1	—
50	S	2.3	4.2	54.7	1	—
55	S	2.85	5.1	55.8	2	—
60	S	2.25	7.25	31	1	—
60	S	4	7.2	55.5	1	—
66	S	4.75 -	7.75	61.3	2	4.0
69	S	3.1 +	6.25	49.6	1	3.4
80	S	3.5 -	7.25	48.8	2	3.1
95	S	6.2 +	11.7	53	Female	6.4
96	S	6.3 -	12.1	52	2	5.9
100	S	8 +	14.5	55	2	8.4
100	S	9 +	16.5	54.5	2	9.5
105	S	6.5 -	12.5	52	2	6.1
105	S	8 -	15	53.3	2	6
110	S	7.25 -	16.5	43.9	2	7
118	S	8.4 +	19.5	43.1	2	9.4
120	T	—	—	—	2	—
140	S	9.2 +	23	40	2	9.6
150	S	12.0 -	24.0	50	2	10.5
165	S	10	27	37	2	—
180	S	16.5 +	30	55	2	17
205	S	15 +	34	44.1	Female	16
205	S	22 -	35	62.8	2	18.5
220	S	18 +	38	47.3	2	19
220	S	15	37	40.5	2	—
264	S	20.1 +	43	46.7	Female	24
283	S	26.5 -	55	48.1	2	25
300	S	31 ±	60	51.6	2	31
300	S	25 +	55	45.4	3	27
330	S	31.5 -	65	48.5	3	30
350	S	35	73	47.9	Female	—
390	S	34 +	104	52.2	3	40
Adult	—	890	1773	50.1	Male	—
		732	1673	43.75	Male	—
		850	1800	47.2	Male	—
		755	1750	42.8	Male	—
		655	1761	46.5	Male	—
		710	1520	46.7	Male	—
		925	1855	51	Male	—
		900	1800	50	Female	—
		730	1430	51	Female	—
		700	1243	56.3	Female	—

S=sagittal; T=transverse.

Note. In column (3), the sign + or - or ± indicates whether the pouch is above (+) or below (-) or level with (±) Houston's main valve. In column (6), the numbers 1, 2, 3, indicate whether the relationship of the pouch anteriorly is below ejaculatory duct (1), above ejaculatory duct, but below neck of bladder (2), or above the neck of the bladder (3). Measurements in the adult pelvis were carried out in the post-mortem room.

opening of the Wolffian ducts into the urethra. During the succeeding months it comes to lie behind the prostate, and during the last month it is usually situated behind the bladder. But the apparent upward migration of the pouch is belied by the relationship to Houston's main valve. From the time when this valve can be recognized (fourth month onwards) the pouch is always situated in its immediate vicinity. Frequently, even during the fourth month (at the time when according to Elliot Smith the major upward migration has yet to take place), the pouch is found not below the main valve, as would have been expected, but above it (see Pl. 1, fig. 5).

These two sets of observations appear to be contradictory, and it might be argued that the rectum or its valves could migrate upwards and keep pace with the upward movement of the pouch. This is extremely unlikely, because in the foetus the valves of Houston frequently lie higher in relation to the pieces of the sacrum than they do in the definitive position. The tendency will therefore be for the rectum to move downwards rather than upwards.

3. Relation of the pouch to the sacral promontory and perineal surface

In Table 1 the distances promontory-to-pouch are expressed as a percentage of the corresponding distances promontory-to-perineum. Similar measurements taken from ten adult pelves are also shown.

These figures bring out the fact that during the early stages of development (especially up to about the 15 mm. stage) the pouch lies close to the perineal surface. Thereafter it is separated from it by an increasing distance, until by the fourth month the ratio promontory-to-pouch/promontory-to-perineum falls within the limits observed in the adult.

As has already been explained, the reduction in this ratio does not indicate whether the pouch has migrated upwards or whether the perineal surface has migrated downwards, or whether both factors are involved. Even if it is agreed that upward migration occurs during the first 3 months of pregnancy, the figures do not support the view that the pouch migrates upwards from the fourth month onwards. These findings are therefore in agreement with the observations concerning the relations of the pouch to Houston's main valve.

4. The position of the bladder

The bladder, in the embryo and foetus, is seen to lie mainly in the abdomen. We are concerned here with the position of the neck of the bladder and prostate only, since these are the only parts which come in contact with the pouch. Pl. 1, fig. 8, shows the appearance of the medial true ligament of the bladder. The vertical direction of the ligament is demonstrated. It is interesting to notice the presence of many muscle fibres in the ligaments at this stage. During the last month of pregnancy the ligaments take up a horizontal position, and at the same time the neck of the bladder descends. This downward movement is shown in Pl. 1, figs. 3 and 7. Pl. 1, fig. 3, shows the appearance of a sagittal section of the pelvis in a 300 mm. foetus; a seeker has been placed in the ureteric orifice. The pouch is related to the prostate, and (not shown in figure) the true ligaments of the bladder run vertically upwards. Pl. 1, fig. 7, shows a similar section of a 330 mm. foetus; a seeker has again been placed in the ureteric orifice. The bladder has descended in the latter

specimen in which the true ligaments (not shown) run horizontally. From the figures, and from Table 1, it can be seen that the distance from the pouch to the perineum in the two cases is almost identical, but that whereas in the 300 mm. specimen the pouch is related to the prostate, in the 330 mm. foetus the pouch is related to the bladder. It may also be noted that the relationship to Houston's main valve remains unchanged.

It is well known that the bladder descends from the abdomen into the pelvis during infancy and childhood (Symington, 1887). The evidence presented here merely shows that this downward movement begins shortly before birth.

5. *The septum recto-vesicale*

The septum recto-vesicale is first clearly seen during the fourth month. A condensation of areolar tissue appears near the midline between rectum and prostate. At this stage the recto-urethralis muscles are very well developed (Pl. 1, fig. 5), and the lower part of the septum is lost in the angle between the superior recto-urethralis and the urethra. As the septum is traced upwards it is joined by fibres which are derived from the rectum (Pl. 1, fig. 5). Great interest attaches to these fibres whose continuity with the rectum does not appear to have been described before. Beginning below the lowest of Houston's valves, they pass upwards and forwards spraying out into finer and finer fibres as they do so, until ultimately they are lost in the dense connective tissue in which the prostatic tubules and the ampullae of the vasa deferentia are embedded. During the later stages the delicate condensation of areolar tissue develops into a definite fascial plane in which these muscle fibres are incorporated. Muscle fibres occur also in the recto-vaginal septum (Pl. 1, fig. 6).

Since the septum has already appeared by the fourth month, before the major alteration in the relations of the pouch has taken place, it seems unlikely that it is formed in the manner postulated by Elliot Smith.

CONCLUSION

No direct evidence has been found showing that the peritoneum contributes to the formation of the septum recto-vesicale. Any theory of the formation of the septum must take into account the presence of muscle fibres which are continuous with the longitudinal muscle coat of the rectum. The simplest explanation seems to be that the septum is formed in a manner similar to many other fascial septa in the body, i.e. by the condensation of areolar tissue.

SUMMARY

1. A review of the literature shows three radically different accounts of the fate of the peritoneum lining the recto-vesical pouch and of its role in the formation of the septum recto-vesicale (of Denonvilliers).
2. Fifty-two embryos and fetuses have been examined, covering the period from the end of the first month to term.
3. The position of the pouch has been considered, not only in relation to the prostate anteriorly, but also to the rectum posteriorly, to the surface of the perineum inferiorly, and to the sacral promontory superiorly.

4. The times of appearance and histological features of the septum recto-vesicale have been examined.

5. The findings are compatible with upward migration of the pouch during the first 3 months of pregnancy, but no evidence of peritoneal fusion has been found. No evidence of upward migration was found after the fourth month. An important factor in producing change in the anterior relations of the pouch is the descent of the neck of the bladder, which occurs during the last month of foetal life. There is no evidence that the septum recto-vesicale receives a contribution from the peritoneum; on the contrary, it has been shown to contain muscle fibres which are continuous with the external longitudinal muscle coat of the rectum. The septum appears to be formed simply by the condensation of loose areolar tissue.

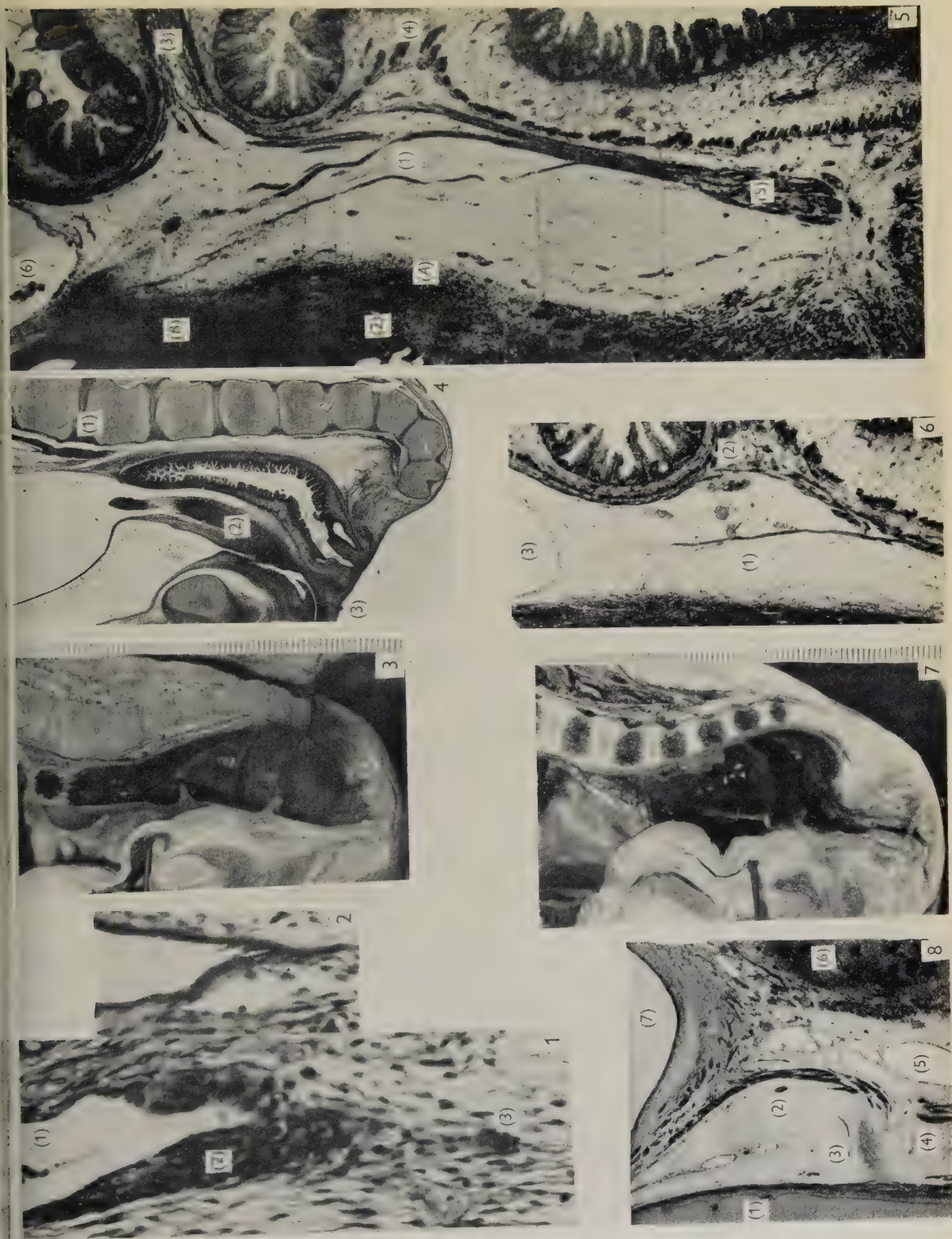
I wish to thank Prof. E. W. Walls for many valuable suggestions, Prof. J. D. Boyd for kindly allowing me to examine his collection of embryos, and Mr P. A. Runnicles for the histological preparations and photography. Finally I wish to thank the Central Research Fund of the University of London for the purchase of a microtome, and Miss P. G. Evans for help with the manuscript.

REFERENCES

- BORGHESE, E. (1937). *La morfogenesi de connettivo perivesicale*. Turin: Rosenberg and Sillier.
- BUCHANAN, A. M. (1949). *Manual of Anatomy*, 8th ed. by F. W. Jones. London: Baillière, Tindall and Cox.
- CUNEO, B. & VEAU, V. (1899). De la signification morphologique des aponeuroses périvésicales. *J. Anat., Paris*, **35**, 235-245.
- CUNNINGHAM, D. J. (1951). *Textbook of Anatomy*, 9th ed. by J. C. Brash. London: Oxford University Press.
- DENONVILLIERS, C. P. (1836). Anatomie du périnée. *Bull. Soc. Anat., Paris*, 3rd Series, **2**, 105-106.
- EBERT, J. D. (1956). Director, Laboratory of Embryology, Carnegie Inst. of Washington. Personal communication.
- GRAY, H. (1954). *Anatomy, Descriptive and Applied*, 31st ed. London. Longmans, Green and Co.
- KEITH, A. (1914). The nature of peritoneal adhesions. *Lancet*, **2**, 362-365.
- KEITH, A. (1946). *Human Embryology and Morphology*, 6th ed. London: Edward Arnold and Co.
- SILVER, P. H. S. (1955). Observations on the valves of Houston in the human embryo and foetus. *J. Anat., Lond.*, **89**, 217-224.
- SMITH, G. ELLIOT (1908). Studies in the anatomy of the pelvis with special reference to the fasciae and visceral supports. *J. Anat., Lond.*, **42**, 198-218, 252-270.
- SYMINGTON, J. (1887). *The Anatomy of the Child*. Edinburgh: E. and S. Livingstone.
- TOBIN, C. E., & Benjamin, A. (1945). Anatomical and surgical restudy of Denonvilliers' fascia. *Surg. Gynec. Obstet.* **80**, 373-388.
- UHLENHUTH, E., WOLFE, W. M., SMITH, E. M., and MIDDLETON, E. B. (1948). Recto-genital septum. *Surg. Gynec. Obstet.* **86**, 148-163.
- WESSON, M. B. (1922). The development and surgical importance of the recto-urethralis muscle and Denonvilliers' fascia. *J. Urol.* **8**, 339-359.
- WESSON, M. B. (1923). Fasciae of the uro-genital triangle. *J. Amer. Med. Ass.* **81**, 2024-2030.

EXPLANATION OF PLATE

- Fig. 1. Sagittal section of 39 mm. embryo (81:183:2) to show true peritoneal fusion occurring between the mesentery of the third part of the duodenum (to right of figure) and the posterior abdominal wall (to left of figure). $\times 100$. (1) Thin layer of normal peritoneum. (2) Thickening of true fusion. (3) Island of peritoneal cells now submerged in extraperitoneal connective tissue.
- Fig. 2. Sagittal section of 55 mm. embryo (55:117:1) to show artefact at the bottom of the recto-vesical pouch, which may be mistaken for true fusion. Compare with fig. 1. $\times 100$.
- Fig. 3. Naked-eye appearance of sagittal section of pelvis of male foetus 300 mm. C.R.L. (E. 13a). Scale in mm. The pouch lies behind the prostate approximately level with Houston's main valve. Seeker in ureteric orifice at the level of the upper border of the symphysis.
- Fig. 4. Sagittal section of pelvis of male embryo 55 mm. C.R.L. (end of third month) (55:107:1). $\times 9$. (1) Sacral promontory. (2) Recto-vesical pouch. (3) Perineal surface. These three points are approximately in a straight line. The bladder is distended and the rectum contains meconium. The m. recto-urethralis superior can be seen, lying far below the recto-vesical peritoneal pouch.
- Fig. 5. Sagittal section of male foetus 100 mm. C.R.L. (end of fourth month: no. 176). Composite figure of three sections (100:2, 103:2, 105:2). $\times 28$. Notice the presence of muscle fibres derived from the rectum. (1) Muscle fibres lying in septum recto-vesicale. (2) Prostate and prostatic urethra. (3) Houston's main valve. Notice relation *below* recto-vesical pouch. (4) Houston's lowest valve. (5) M. recto-urethralis superior. (6) Recto-vesical pouch.
- Fig. 6. Sagittal section of pelvis of female foetus 95 mm. C.R.L. (177:103:1) to show that in the female muscle tissue also occurs in the septum recto-vaginalis. $\times 23$. Compare with fig. 5. (1) Muscle fibres derived from rectum. (2) Houston's lowest valve. (3) Recto-vaginal pouch.
- Fig. 7. Naked-eye appearance of sagittal section of pelvis of male foetus 330 mm. C.R.L. (E. 13). Scale in mm. The pouch lies behind the bladder (compare fig. 3), and is still level with Houston's main valve. Seeker in ureteric orifice at the level of the middle of the body of the pubis.
- Fig. 8. Parasagittal section of pelvis of male foetus 100 mm. C.R.L. (176:52:1) to show the vertical disposition of the medial true ligament of the bladder. $\times 28$. (1) Pubis. (2) Medial true ligament of the bladder. (3) White line. (4) Levator ani. (5) Dorsal vein of penis joining prostatic venous plexus. (6) Prostate with prostatic urethra cut tangentially. (7) Bladder lumen.



SILVER—PERITONEUM IN THE FORMATION OF THE SEPTUM RECTO-VESSICALE

(Facing p. 546)

CORNIFICATION OF THE HUMAN VAGINAL EPITHELIUM

By A. W. ASSCHER, C. J. TURNER

The London Hospital Medical College, London, E.1

AND C. H. DE BOER

The Women's Hospital, Liverpool

INTRODUCTION

Many authors have described the cyclical changes in the human vaginal epithelium, but the cornification of this epithelium has received less precise attention. It is the purpose of this investigation to study the cornification of the human vaginal epithelium by histochemical and other techniques.

Dierks (1927) considered that cornification occurred in an intra-epithelial zone, the 'Verhornungszone' (zone of cornification), but Stieve (1931*a, b*) maintained that the appearance of this zone resulted from mechanical factors. Stemshorn (1928) considered that there was insufficient evidence for the occurrence of cornification in the intra-epithelial zone; he thus proposed the non-committal term 'Verdichtungszone' (zone of densification). Traut, Bloch & Kuder (1936) described the occurrence of cornification in the superficial layers of the epithelium; during the menstrual cycle no consistent changes in these layers were observed. Papanicolaou, Traut & Marchetti (1948) also described cornification of the superficial portion of the epithelium; they recognized the existence of an intra-epithelial zone which was not always well defined and which often gave the impression of an artefact produced by shrinkage of the epithelium. Papanicolaou (1954) stated that the functional significance of this zone is not yet properly understood.

MATERIAL AND METHODS

Twenty-eight biopsy specimens, taken from different subjects at different stages of the menstrual cycle, were used. The subjects included in this investigation showed no evidence of unusual hormonal balance; in eleven cases an endometrial biopsy, taken at the time of the vaginal biopsy, revealed a normal endometrium corresponding in its development to the stage of the cycle determined from the menstrual history. All specimens were taken from the anterior vaginal wall at a level just above the bladder neck; they were immediately fixed in a solution containing 1 g. of trichloroacetic acid dissolved in 100 ml. of 80% ethyl alcohol. The specimens were embedded in paraffin; serial sections, cut at 7 μ , were prepared and studied with the following procedures:

- (i) Ehrlich's haematoxylin and eosin.
- (ii) Heidenhain's haematoxylin.
- (iii) Michrome (M.F.4) stain (Edward Gurr, Ltd.). The vaginal smear staining technique described by Gurr (1953) was adapted for use on sections by lengthening the staining period to 5 min.

(iv) Papanicolaou's vaginal smear stain (E.A.36). The modified technique described by Papanicolaou *et al.* (1948) was used; it was adapted for use on sections by lengthening the staining periods in O.G.6 and E.A.36 to 5 min. each.

(v) Inspection of unstained material with polarized light.

(vi) Barrnett & Seligman (1952, 1954) procedure for the demonstration of sulphhydryl and disulphide groups. Disulphide groups were localized by comparing preparations, previously reduced for 1 hr. with 0.5M thioglycollic acid at a pH of 8.5 and a temperature of 37° C., with corresponding sulphhydryl group preparations. Controls were incubated for 1 hr. at 50° C. in 0.1M iodoacetic acid at pH 8.

OBSERVATIONS

(a) *The follicular phase epithelium*

Observations were made on ten specimens taken from the 3rd to the 12th day of the menstrual cycle. The deepest six to ten layers of the epithelium show sulphhydryl group positivity of the cellular cytoplasm and intercellular bridges. The superficial portion of the epithelium displays sulphhydryl group positivity of the cell walls. An intermediate zone, consisting of flattened acidophilic cells, can frequently be distinguished; its sulphhydryl group positivity appears greater than that of the overlying superficial cells. This appearance might be attributable to an optical artefact resulting from the flattening of its cells. The intermediate zone is further distinguished by the presence of sulphhydryl groups in the peripheral cytoplasm of its constituent cells. This intermediate zone shows greater contrast to the remainder of the epithelium after thioglycollic acid reduction, indicating the presence of disulphide groups that are localized in the cell walls and peripheral cytoplasm (Pl. 1, figs. 1, 3). This zone can be shown to contain a considerable amount of birefringent material (Pl. 1, fig. 4). The intermediate zone shows great affinity for Heidenhain's haematoxylin, acquiring an intense black colour that frequently extends throughout the cytoplasm (Pl. 1, fig. 2). The intermediate zone assumes an intense yellow coloration with Michrome vaginal smear stain (Pl. 2, fig. 5), whereas it acquires a red coloration with Papanicolaou's stain (Pl. 2, fig. 6). The thickness of the intermediate zone varies in different regions of the same specimen, its average thickness, however, is related to that of the whole epithelium. Thus, since the vaginal epithelium grows in thickness during the follicular phase (Papanicolaou *et al.* 1948), it may be inferred that the intermediate zone also increases in thickness during this phase.

(b) *The mid-cycle epithelium*

Observations were made on seven specimens taken between the 12th and 17th days of the menstrual cycle. A disulphide-containing, birefringent zone was found on the surface of the epithelium in places in five of these specimens (Pl. 2, fig. 7), whilst elsewhere this zone was covered by a few layers of cells in which no disulphide groups could be demonstrated. The disulphide-containing, birefringent zone in the remaining two specimens, taken on the 15th and 17th days of the cycle, was found on the surface of the epithelium throughout the specimen. In the superficial zones of these latter specimens disulphide and sulphhydryl groups were not merely

confined to the cell walls and peripheral cytoplasm; some of the cells showed disulphide and sulphydryl groups throughout their cytoplasm. Furthermore, in these specimens the superficial zone assumed a more intense crimson coloration with the Barrnett and Seligman procedure, particularly after thioglycollic acid reduction. This superficial zone showed with all other techniques employed properties identical to those of the intermediate zone of the preceding follicular phase.

(c) *The luteal phase epithelium*

Eleven specimens taken between the 17th and 28th days of the menstrual cycle were studied. Early luteal-phase specimens showed a disulphide-containing, birefringent zone on the surface of the epithelium; its tinctorial properties with the other techniques employed were similar to those of the intermediate zone of the follicular phase. Some cells in this zone contained disulphide and sulphydryl groups throughout their cytoplasm, in others they were confined to the cell walls and peripheral cytoplasm (Pl. 2, fig. 8). The superficial zone acquired an intense crimson appearance with the Barrnett & Seligman procedure in these specimens, as in the two mid-cycle specimens. A considerable individual variation was found in the thickness of the superficial zone during the early luteal phase.

Late luteal phase specimens did not show the disulphide-containing, superficial zone (Pl. 2, fig. 9). A zone of flattened cells could occasionally be distinguished in these specimens; it was intermediate in position and appeared particularly in areas overlying the subepithelial papillae. This zone showed properties similar to those of the intermediate zone of the early follicular phase, but its staining reactions were patchy and less well defined.

An intense scattered yellowness of the deeper layers of the epithelium, extending to the surface in places, was observed in two of the late luteal-phase specimens when stained with Michrome vaginal smear stain (Pl. 2, fig. 10); with Papanicolaou's stain a similar distribution of red coloration was found. These areas were not stained with Heidenhain's haematoxylin and they showed no birefringence or disulphide groups.

DISCUSSION

The functional significance of the intra-epithelial zone of the human vaginal epithelium has been the subject of much discussion. Stieve (1931*a, b*) and Traut *et al.* (1936) attributed no importance to the zone in view of its inconstancy. Stieve pointed out that intra-epithelial zones could be demonstrated at any stage of the menstrual cycle as well as after the menopause and that they could even be found in the buccal and oesophageal mucosae of both male and female subjects. He therefore concluded that its presence was not dependent on endocrine relationships but on mechanical factors.

The present observations show that the follicular-phase human vaginal epithelium possesses a specialized intra-epithelial zone with distinctive chemical and physical properties. Disulphide groups and birefringence are localized in this zone; it shows great affinity for Heidenhain's haematoxylin and can be selectively demonstrated with certain vaginal smear stains. Such properties are unlikely to be those of an artefact; these characteristics of the intra-epithelial zone clearly indicate that it constitutes a site of cornification, thus substantiating the view of Dierks (1927).

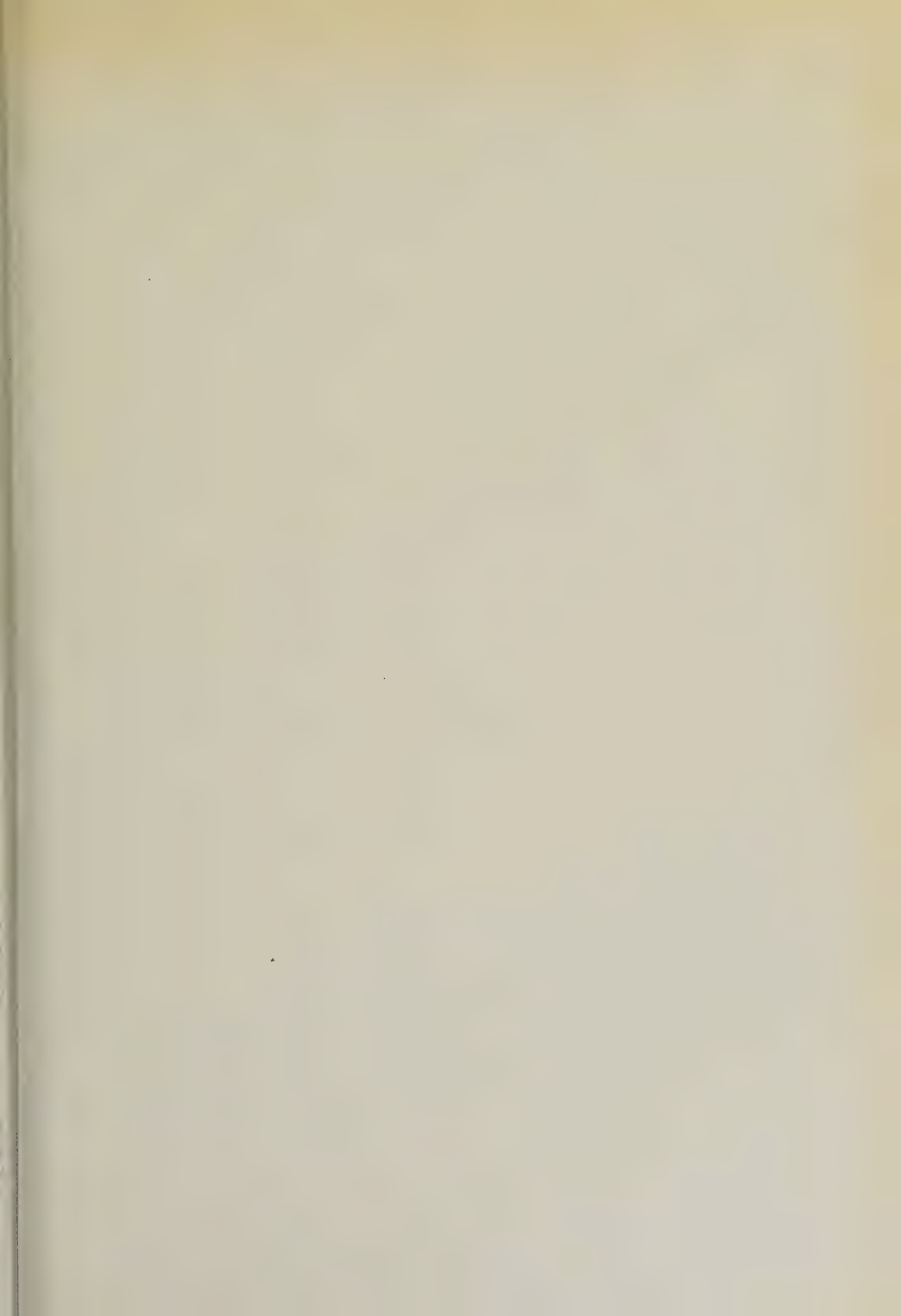
The intra-epithelial zone of the follicular phase closely resembles the intra-epithelial zone of the pro-oestrous mouse vaginal epithelium. Asscher & Turner (1955) showed that during the oestrous cycle of the mouse, disulphide groups first appear in the intra-epithelial zone of the pro-oestrous epithelium. A disulphide-containing, birefringent intra-epithelial zone can be experimentally produced in ovariectomized mice by the administration of oestrogens (Asscher & Turner, unpublished). This comparative histochemical evidence suggests that the existence of an intra-epithelial zone in the human vaginal epithelium might also be dependent on hormonal factors.

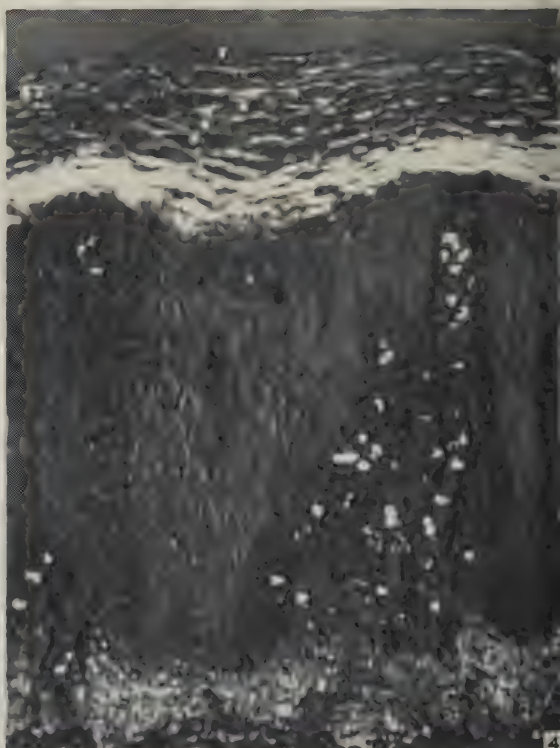
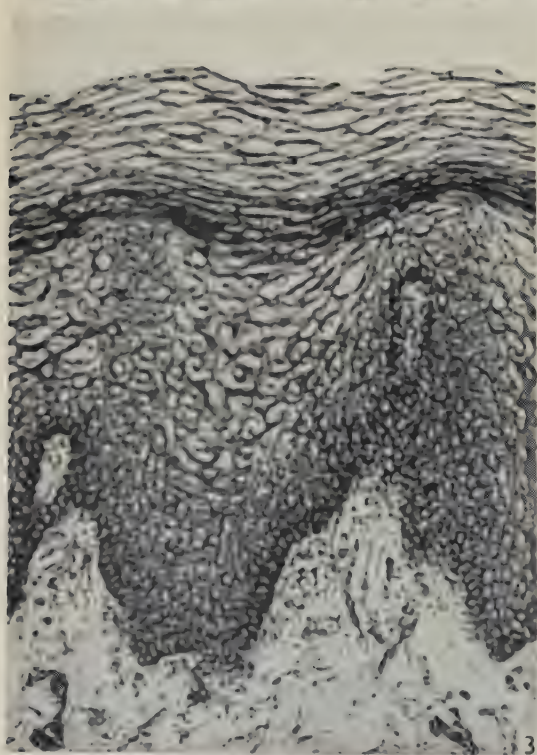
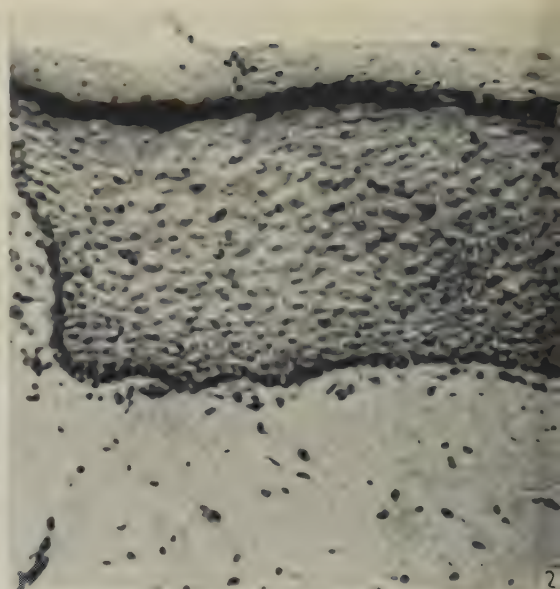
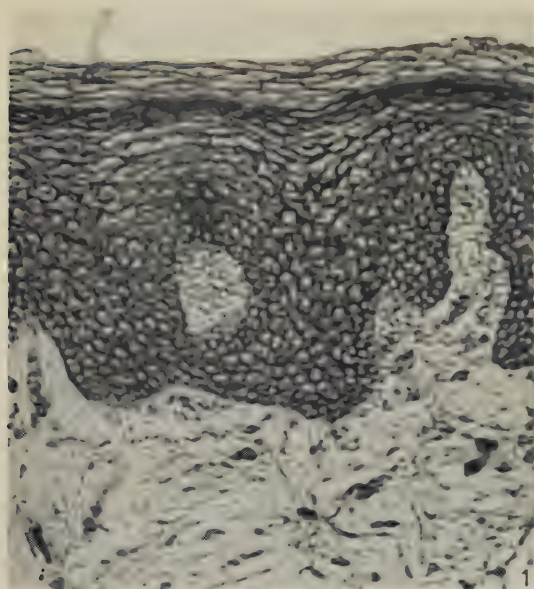
The interpretation of cyclical phenomena from random specimens must necessarily be speculative; thus the present observations can do no more than indicate the following tentative sequence of events in the formation and fate of the intra-epithelial zone during the menstrual cycle. This cornification zone appears to increase in thickness during the follicular phase; it attains a more superficial position towards the middle of the cycle and is found in a completely superficial position during the early luteal phase; at this time it possesses the greatest amount of disulphide groups, as judged from the colorations observed with the Barnett & Seligman technique. This cornification zone appears to be desquamated during the luteal-phase and thus no evidence of it remains at the end of this phase. In late luteal phase specimens evidence of intra-epithelial zone regeneration may frequently be found. The superficial layers of vaginal epithelia may be exposed to a variety of exogenous influences; this may in part account for the variable times at which the cornification zone was found to reach the surface and later to be desquamated. De Allende & Orias (1950) found the highest percentage of cornified cells in vaginal smears taken during the middle of the cycle; the present account of the cornification of the human vaginal epithelium provides a histological basis for this observation, since it is at this stage of the cycle that the cornification zone forms the superficial part of the vaginal epithelium.

The presence of disulphide groups in association with birefringence may be taken to indicate keratin; the use of Michrome and Papanicolaou's vaginal smear stains has produced yellow and red colorations respectively, in all those regions of the epithelium which contained keratin on the basis of the above-mentioned criterion. Red, orange or yellow colorations are considered to indicate the presence of keratin with these stains (Gurr, 1953); the value of these stains in the demonstration of cornified cells in the human vaginal epithelium is thus confirmed. These particular colorations were produced in two specimens, however, in regions in which no keratin could be detected histochemically or by polarized light. It is therefore concluded that some other substance(s) may occasionally imitate the staining reactions of keratin with these vaginal smear stains.

CONCLUSIONS

1. An intra-epithelial zone has been demonstrated in the human vaginal epithelium during the follicular phase. It possesses distinctive chemical and physical properties which indicate that it constitutes a site of cornification. Its development and fate during the menstrual cycle are discussed.





ASSCHER, TURNER AND DE BOER - CORNIFICATION OF THE HUMAN VAGINAL EPITHELIUM

(Facing p. 551)

2. The value of Papanicolaou's (E.A.36) and Michrome (M.F.4) vaginal smear stains in the demonstration of cornified cells in the human vaginal epithelium has been confirmed; anomalous staining reactions were observed in two specimens.

We are greatly indebted to Prof. R. J. Harrison for extending the facilities of his department to us and for critically reading the manuscript; we are also grateful to Prof. W. Montagna for his helpful suggestions. We wish to thank Mr R. Quinton Cox for his care in the preparation of the photographs and Mr D. A. McBrearty and Mr C. A. Saxton for cutting some of the sections. We are grateful to Edward Gurr, Esq. for supplying stains and for defraying the cost of the coloured plate.

REFERENCES

- ASSCHER, A. W. & TURNER, C. J. (1955). Vaginal sulphydryl and disulphide groups during the oestrous cycle of the mouse. *Nature, Lond.*, **175**, 900-901.
- BARNETT, R. J. & SELIGMAN, A. M. (1952). Histochemical demonstration of protein-bound sulphydryl groups. *Science*, **116**, 323-327.
- BARNETT, R. J. & SELIGMAN, A. M. (1954). Histochemical demonstration of sulphydryl and disulfide groups of protein. *J. nat. Cancer Inst.* **14**, 769-804.
- DE ALLENDE, I. L. C. & ORIAS, O. (1950). Trans. G. W. Corner, *Cytology of the Human Vagina*. New York: Hoeber.
- DIERKS, K. (1927). Der normale mensuelle Zyklus der menschlichen Vaginalschleimhaut. *Arch. Gynaek.* **130**, 46-69.
- GURR, E. (1953). *A Practical Manual of Medical and Biological Staining Techniques*. London: Leonard Hill, Ltd.
- PAPANICOLAOU, G. N., TRAUT, H. F. & MARCHETTI, A. A. (1948). *The Epithelia of Woman's Reproductive Organs*. New York: The Commonwealth Fund.
- PAPANICOLAOU, G. N. (1954). *Atlas of Exfoliative Cytology*. Cambridge, Mass.: Harvard University Press.
- STEMSHORN, F. (1928). Zur Frage des menstruellen Zyklus der menschlichen Vaginalschleimhaut. *Zbl. Gynäk.* **52**, 2387-2392.
- STIEVE, H. (1931a). Verhornungserscheinungen im Epithel der menschlichen Speiseröhren- und Scheidenschleimhaut. *Z. mikr.-anat. Forsch.* **24**, 213-236.
- STIEVE, H. (1931b). Über angebliche zyklische Veränderungen des Scheidenepithels. *Zbl. Gynäk.* **55**, 194-201.
- TRAUT, H. F., BLOCH, P. W. & KUDER, A. (1936). Cyclical changes in the human vaginal mucosa. *Surg. Gynec. Obstet.* **63**, 7-15.

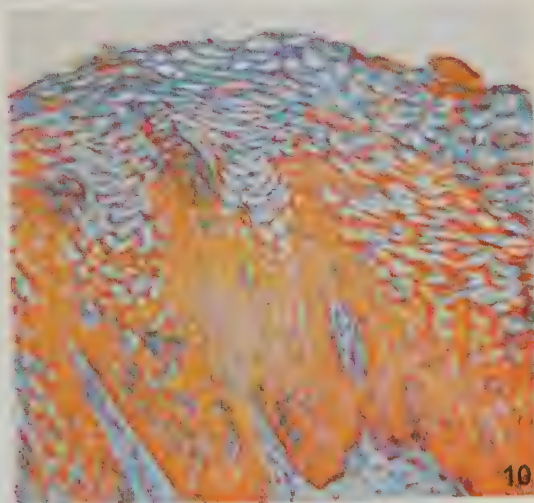
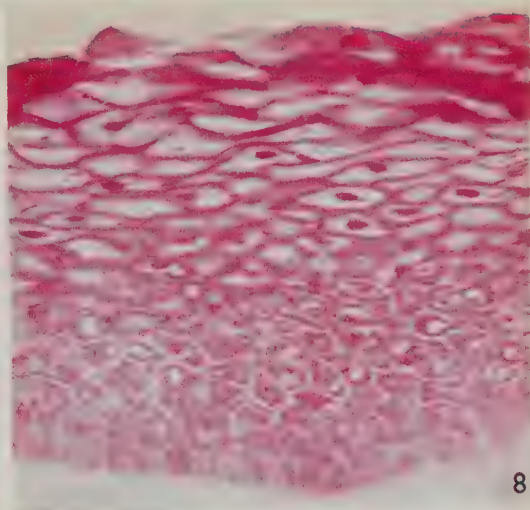
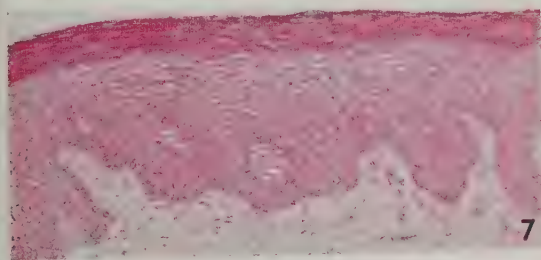
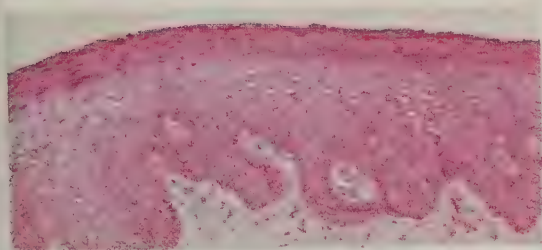
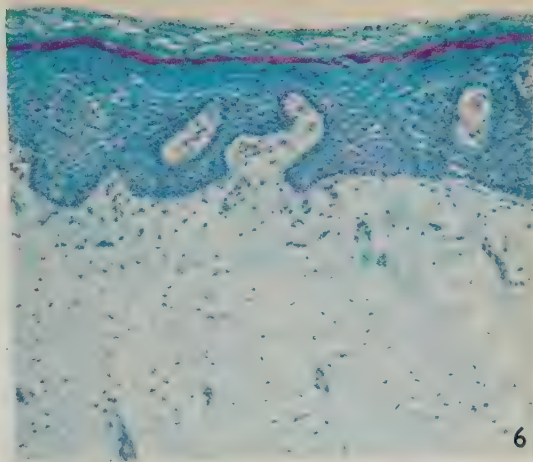
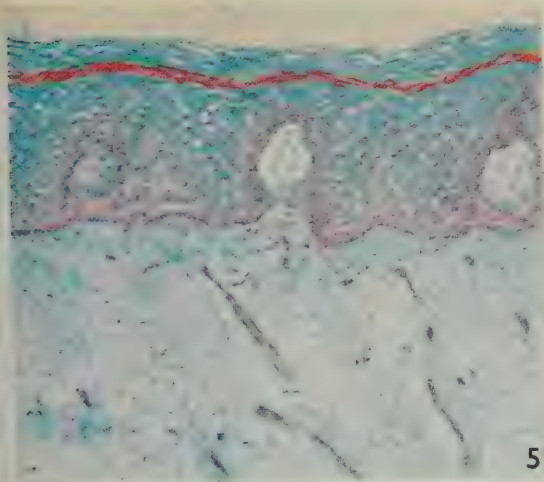
EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Sulphydryl and disulphide group distribution in the human vaginal epithelium on the 8th day of a 28-day menstrual cycle. Barnett & Seligman procedure. $\times 288$. Disulphide groups are localized in the cell walls and peripheral cytoplasm of the cells of the intermediate zone.
- Fig. 2. Same epithelium as shown in fig. 1 stained with Heidenhain's haematoxylin. $\times 288$. The intermediate zone shows an intense black coloration.
- Fig. 3. Sulphydryl and disulphide group distribution in the human vaginal epithelium on the 12th day of a 28-day menstrual cycle. Barnett & Seligman procedure. $\times 288$. The disulphide-containing, intermediate zone shows greater thickness at this stage of the menstrual cycle.
- Fig. 4. Same epithelium as shown in fig. 3 seen under polarized light. $\times 288$. Birefringence is largely confined to the intermediate zone.

PLATE 2

- Fig. 5. Same epithelium as shown in fig. 1. $\times 180$. Stained with Michrome vaginal smear stain (M.F.4). The intermediate zone shows an intense yellow coloration.
- Fig. 6. Same epithelium as shown in fig. 1. $\times 180$. Stained with Papanicolaou's vaginal smear stain (E.A.36). The intermediate zone shows a red coloration.
- Fig. 7. Human vaginal epithelium taken on the 14th day of a 28-day menstrual cycle. Barnett & Seligman procedure. $\times 180$. The upper section shows the distribution of sulphydryl groups alone, the lower section shows sulphydryl as well as disulphide groups. A comparison of these sections, which is facilitated by viewing them through a green filter, reveals that the disulphide-containing zone is in a superficial position in most areas of this specimen.
- Fig. 8. Distribution of disulphide and sulphydryl groups in a specimen taken on the 22nd day of a 28-day menstrual cycle. Barnett & Seligman procedure. $\times 320$. The disulphide-containing zone is completely superficial and shows an intense crimson coloration. Some of the cells of the superficial zone possess disulphide groups throughout their cytoplasm; in others these groups are confined to the cell walls and peripheral cytoplasm. The intercellular bridges and some of the nuclei show sulphydryl group positivity.
- Fig. 9. Human vaginal epithelium taken on the 27th day of a 28-day menstrual cycle. Barnett & Seligman procedure. $\times 180$. No disulphide groups could be demonstrated in this specimen.
- Fig. 10. Same epithelium as shown in fig. 9. $\times 180$. Stained with Michrome vaginal smear stain (M.F.4). Note the intense, patchy yellowness of the deeper layers of the epithelium, extending to the surface in places; in these areas no disulphide groups or birefringence could be demonstrated.



THE ABERRANT RENAL ARTERY

By F. T. GRAVES, M.S., F.R.C.S.

King's College Hospital, London

Aberrant or accessory arteries have been of interest to the clinician for some years, mainly because of the possible part that the vessel may play in the causation of hydronephrosis. Many cases have been described in the literature in which an aberrant or accessory artery is found to enter the lower pole of the kidney and to lie in such a position as to appear to obstruct the outflow of urine at the pelvi-ureteric junction. Schools of thought are divided as to whether the obstruction is in fact due to kinking of the ureter by the vessel, or whether it is due to a so-far unexplained neuromuscular inco-ordination at the pelvi-ureteric junction. Opinion is also divided on the advisability of dividing the aberrant vessel, both as to the effects that this may have upon the obstruction, and on the possibility of resultant necrosis of part of the kidney.

Hitherto, a widely held view has been that normally the kidney is supplied by a single renal artery which enters the hilum and that any additional artery entering the organ at one or other pole is necessarily an extra and aberrant source of supply to that provided by the main stem artery. In 1552 such anomalous or aberrant arteries were recorded by Eustachius in one of his famous plates which remained unprinted in the Papal Library until 1714. Since then many more cases of aberrant renal arteries have been reported. Such vessels entering the upper pole of the kidney arise from either the aorta or a suprarenal artery, whereas those entering the lower pole may stem from the aorta, common or internal iliac, superior mesenteric (Anson, Cauldwell, Pick & Beaton, 1948) or spermatic (Winsbury-White, 1936; Crelin, 1948) artery.

The incidence of aberrant arteries has been variously reported as nearly 50% (Helström, 1927) and 25% (Edsman, 1954). However, judging by the many descriptions of these vessels in the literature, it is evident that there is no established criterion for aberrance; the term has been applied equally to an additional artery in the renal pedicle, or to a vessel entering the kidney at either pole, whether derived from the main renal artery, from the aorta or from a branch of the aorta.

In order to clarify the situation, both from the viewpoint of the anatomist and the surgeon, it is necessary to consider two questions. First: What contribution do accessory or aberrant arteries make to the blood supply of the kidney? Secondly: How can the presence of aberrant vessels, and their range of pattern, be explained?

THE ARTERIAL SEGMENTS OF THE KIDNEY

Graves (1954) demonstrated that the distribution of the arteries within the kidney substance was constant and that upon this was founded the division of the renal parenchyma into five segments. These segments were named the apical, upper, middle, lower and posterior segments. The middle and upper segments lie in the

anterior plane of the kidney, the posterior lies in the posterior plane. The apical and lower segments, however, differ from the remainder in that they occupy areas in both the anterior and posterior planes.

The main stem of the renal artery divides at any point between the aorta and the hilum into an anterior and posterior division. The latter continues to supply the posterior segment, the former supplies the remaining segments including the apical segment, although occasionally this segment may receive its blood supply from the posterior division.

The artery to the apical segment is very variable in its origin. Most commonly, however, it arises from the anterior division. In order of frequency it arises from the anterior division or the artery to the upper segment (type I), from the junction of the anterior and posterior divisions (type II), from the main stem renal artery or the aorta itself (type III), and least commonly from the posterior division (type IV). In those cases where the artery to the apical segment arises from the aorta or from the main renal artery near its origin from the aorta, the vessel frequently makes an extra-hilar entry into its segment, and in this respect may have the superficial appearance of being an aberrant artery to the upper pole. Commonly, when the artery to the apical segment enters the segment outside the hilum it does not divide into smaller branches until it has entered its segment. Occasionally, however, the artery divides into a leash of vessels outside the kidney (Text-fig. 2; type IIIc), so that each member of the leash enters the segment separately.

For the details of the blood supply to the other four segments Graves (1954) should be consulted.

THE PATTERN OF THE BLOOD SUPPLY

Experiments were carried out which proved that there was no collateral arterial circulation between the segments of the kidney. Not only were plastic casts used but also renal and segmental renal angiography in which more than sixty arteriograms were made. It was thereby shown that ligation of the artery to a segment, or a subsegment, would produce ischaemia of the area supplied by that vessel.

THE NATURE OF ABERRANT ARTERIES

Study of casts of the renal arterial system has shown that if the arteries of the renal pedicle are examined in conjunction with the intrarenal arteries, and not as separate entities, it becomes evident that the so-called aberrant or accessory arteries are in fact normal segmental arteries whose origin is more proximal than usual. This proximal origination accounts for many of the cases of multiple or supernumerary arteries of the renal pedicle.

In those cases where additional arteries are present, those to the lower pole appear to be the most frequent. Weinstein, Countiss & Derbes (1940) found that the incidence of accessory arteries passing to the lower pole was approximately twice that passing to the upper pole, and similar figures were recorded by Albarran (1909).

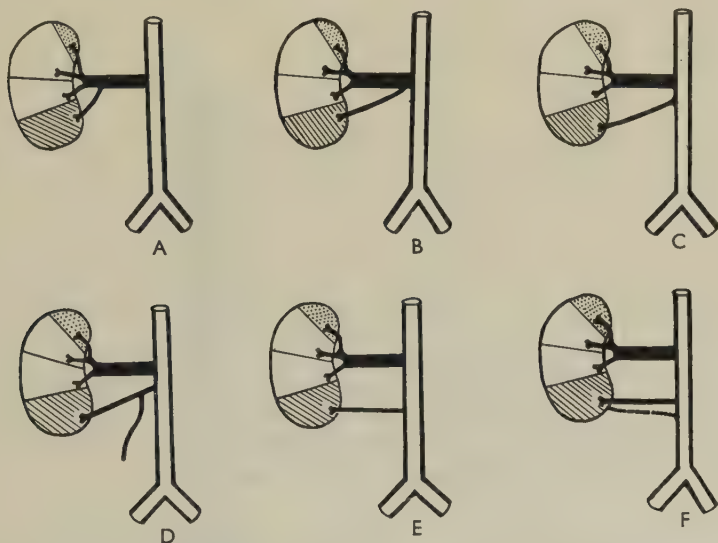
In 63% of the casts examined the artery to the lower segment has the most proximal origin of all the segmental arteries.

VARIATIONS OF THE SEGMENTAL ARTERIES

The variations of the segmental arteries of the kidney appearing as 'aberrant arteries' can be classified into three groups—at the hilum, in the pedicle, from the aorta.

Variations at the hilum

The artery to the lower segment is the only vessel originating at the hilum whose course may appear unusual, in that sometimes in cases of group I (Graves, 1954) the artery may be unusually long and winding in its course before it enters the lower segment (Text-fig. 1A).



Text-fig. 1. A diagram illustrating the variations of origin of the artery to the lower segment of group I. (A) The artery arises at the hilum; (B) from the main stem at its junction with the aorta; (C) from the aorta close to the main stem; (D) from the aorta, the testicular or ovarian artery arises from the artery to the lower segment; (E) the lower segment artery arises from the aorta at some distance from that of the main stem; and (F) the lower segment artery and its posterior branch arise separately from the aorta.

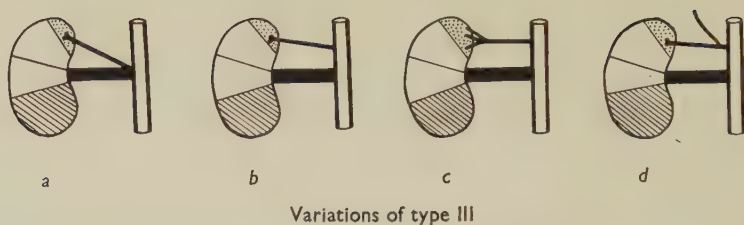
Variations in the pedicle

The presence of more than one artery in the pedicle may be due to:

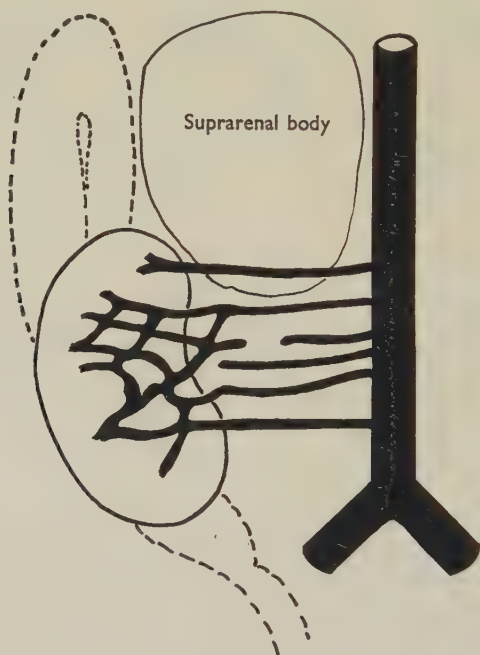
Early division of the main stem at a point close to the aorta, into anterior and posterior divisions (Pl. I, fig. 1).

Apical segment artery arising from the junction of the main stem with the aorta, as in type IIIa (Text-fig. 2a).

Lower segment artery arising close to, or from the junction of the main stem with the aorta (Text-fig. 1B).



Text-fig. 2. A cast of the right kidney seen from the front. The artery to the lower segment arises from the aorta and shortly after its own origin it gives rise to the testicular artery. It corresponds to type D of group I as shown in Text-fig. 1. The artery to the apical segment arises from the main stem as in type III.



Text-fig. 3. A diagram after Felix. This shows the mesonephric arteries, of which some are degenerating. The diagram indicates how persistence of the mesonephric arteries could account for the variation of origin of the adult segmental arteries from the aorta.

Variations from the aorta

The most common arrangement in this group is that of a single artery arising from the aorta together with a normal main trunk, the artery to the lower segment being the most frequent example. A case with multiple aortic origins, as in Pl. 1, fig. 2, is rare, this being the only one found in 70 specimens. The arteries which may arise directly from the aorta are:

Anterior and posterior divisions (Pl. I, fig. 2).

Artery to the apical segment. These are variants of type III (Text-fig. 2). The artery may arise directly from the aorta above that of the main stem and enter its segment at once (type III*b*) or break into a leash of vessels each of which enters the segment separately (type III*c*). It may arise jointly with, or from, the suprarenal artery (type III*d*).

Artery to the lower segment. This can arise directly from the aorta (Text-fig. 1C) at a point just below that of the main stem; it may arise with or give origin to the ovarian or testicular artery (Pl. I, fig. 3 and Text-fig. 1D). The artery may arise from the aorta at a much lower level (Text-fig. 1E). Moreover, as shown in Pl. I, fig. 1 and Text-fig. 1F, its posterior branch may arise direct from the aorta and pass behind the ureter to enter the posterior part of the segment. Usually the posterior branch arises from the lower segment artery close to, and passes under, the inferior calyx to reach the posterior aspect of the lower pole.

DISCUSSION AND SUMMARY

The proximal origin of the segmental arteries of the kidney can be explained by reference to the account of their development given by Felix (1912), see Text-fig. 3.

The mesonephric arteries extend from the sixth cervical to the third lumbar segments. The more cranial of these vessels disappear before those at the caudal end have reached their maximal development. In the upper lumbar region the mesonephric arteries form a network, the rete arteriosum urogenitale from which the reproductive gland, the mesonephros and later the metanephros are supplied with arterial segments. Eventually some of the roots which supply this network degenerate, the area which they supply being taken over by a neighbouring root. This arrangement may explain why those arteries which persist to form the segmental arteries of the adult kidney have some variation in their point of origin.

I suggest that those arteries which have a proximal origin at the hilum or in the pedicle are probably the result of a variation in the degeneration of the rete arteriosum, whilst those which arise from the aorta are persistent mesonephric arteries.

It has been shown that there are five segments of the human kidney, each of which has its own artery and between which there is no collateral circulation. Investigation has shown that the so-called aberrant or accessory arteries are in fact normal segmental arteries whose origin is more proximal than usual.

It is suggested that the artery in question should be named together with an indication of its origin and type as, for instance, 'The Lower Segment (aortic) Artery', and that the words 'aberrant' and 'accessory' which are in present use should be avoided, as they have little anatomical, surgical or descriptive value.

I wish to thank Sir Cecil Wakeley, Bt., for his encouragement and kindly interest in this work.

REFERENCES

- ALBARRAN, J. (1909). *Médecine opératoire des Voies urinaires*, p. 42. Paris: Masson.
- ANSON, B. J., CAULDWELL, E. W., PICK, J. W. & BEATON, L. E. (1948). The anatomy of the pararenal system of veins with comments on the renal arteries. *J. Urol.* **60**, 714-737.
- CRELIN, E. S. (1948). An unusual anomalous blood vessel connecting the renal and internal spermatic arteries. *Anat. Rec.* **102**, 205-211.
- EDSMAN, G. (1954). Accessory vessels of the kidney and their diagnosis in hydronephrosis. *Acta Radiol., Stockh.*, **42**, 26-32.
- EUSTACHIUS, B. (1714). *Tabulae Anatomicae*. Tab. Tertius, Ed. Lancisi: Rome.
- FELIX, W. (1912). *Human Embryology*, ed. Keibel and Mall, vol. 2, fig. 572 and p. 822. Philadelphia: Lippincott.
- GRAVES, F. T. (1954). The anatomy of the intrarenal arteries and their application to segmental resection of the kidney. *Brit. J. Surg.* **42**, 132-139.
- HELLSTRÖM, J. (1927). A contribution to the knowledge of the relation of abnormally running renal vessels to hydronephrosis, and an investigation of the arterial conditions in fifty kidneys. *Acta chir. scand.* **61**, 289-330.
- WEINSTEIN, B. B., COUNTISS, E. H. & DERBES, V. J. (1940). The renal vessels in 203 cadavers. *Urol. cutan. Rev.* **44**, 137-139.
- WINSBURY-WHITE, H. P. (1936). Observations on hydronephrosis with special reference to aberrant vessels. *Trans. Amer. Ass. gen.-urin. Surg.* **29**, 381-401.

EXPLANATION OF PLATE

- Fig. 1. A cast of the right kidney seen from behind. This shows an unusually proximal origin of the anterior and posterior divisions. The main stem is only a quarter of an inch long.
- Fig. 2. A cast of the right kidney seen from the front. Four vessels arise directly from the aorta. From above downwards these are: the posterior division (a pale vessel), the anterior division supplying the upper and middle segments, the anterior branch of the lower segment, the posterior branch of the lower segment. The artery to the apical segment arises from the posterior division, being of type IV.
- Fig. 3. A diagram of the variations of type III of the artery to the apical segment. (a) The artery arises from the main stem close to the aorta; (b) from the aorta; (c) from the aorta but dividing into a leash of vessels each of which enters the segment separately; and (d) from the aorta, the suprarenal artery arising from it.



REVIEWS

Embryologie. Ein Lehrbuch auf allgemein biologischer Grundlage. By D. Starck. 1955. (Pp. xx+688; 502 illustrations, some in colour—and an appendix of Tables; Lex. 8°, D.M. 78.) Stuttgart: Georg Thieme Verlag.

This new text-book of Embryology will be of interest to all biologists whose work is concerned with human and comparative development; the treatment of the subject is along conventional lines in that the work is divided into two sections, general and special. The former covers the period from the origin of the germ cells to the laying down of organ rudiments and the formation of the foetal membranes and placenta. The emphasis here is along comparative lines. The special section on organogenesis, on the other hand, is concerned chiefly with the mammals and man, although in appropriate places illustrations from lower forms are presented and the importance of them is discussed.

It is not the intention of this reviewer to discuss each chapter of the book; some points which have impressed him may be mentioned.

The general section is very wide in its scope and the subjects of genetics and experimental embryology are given full consideration; attention is drawn to the understanding which such work can give in the causation of certain human abnormalities. The section on implantation and placentation is comprehensive, but it is unfortunate that some of the microphotographs illustrating it seem to have suffered during reproduction; of original contributions one was not impressed with the three photomicrographs in fig. 215 selected to show stages in the menstrual cycle. The line drawings in this section are good.

As already mentioned, the section on special development of the organ systems is primarily concerned with the human embryo, and presents a well-balanced account of the various events in the different body systems. One must still make the comment that many of the microphotographs are unsatisfactory. Some have been retouched without adding greatly to their clarity, particularly those in the section on the development of the eye. The line drawings are very clear in the majority of cases, but a few are technically not good. It would be helpful if some drawings could be added in a future edition to elucidate figs. 399 and 421; fig. 433 could be improved, and an obvious error has been made in the labelling of fig. 465*f*, the subcardinal segments of the adult veins having been designated as supracardinals. This is not the usual interpretation.

The tables in the Appendix give information with regard to the relation of body weight of the newborn to the weight of the mother and, in mammals, the duration of pregnancy compared with the number of young. The data presented here are useful.

One can comment with pleasure that the publishers have produced this book in an excellent format. The printing and paper leave little to be desired and it is rather unfortunate that some of the reproductions are not to this same standard.

The book can, however, be recommended to all biological workers.

J. S. BAXTER

Das Zwischenhirn-Hypophysensystem. By W. BARGMANN. (D.M. 28.60.) Berlin: Springer-Verlag, 1954.

Prof. Bargmann, of the Institute of Anatomy in the University of Kiel, is one of the foremost exponents of the view that the hypothalamus is not only a co-ordinating centre of the autonomic nervous system, but also an organ of internal secretion. His main contribution to this concept was made in 1949, when he found that Gomori's chrome-alum haematoxylin-phloxine stain could be specifically used to reveal cytoplasmic inclusions, of an apparently secretory nature, in the cell-bodies and axons of the nuclei supraopticus and

paraventricularis. Since then his Department has become a leading centre for anatomical and experimental research in this field; and Prof. Bargmann a familiar and welcome figure at international conferences at which the subject is discussed.

His monograph, *Das Zwischenhirn-Hypophysensystem*, is a review of the evidence dealing with the relationship of the hypothalamus to the neurohypophysis and adenohypophysis, and begins with a general account of the development and structure (including micro-structure) of the diencephalo-hypophysial system. The next section considers the anatomy of the hypothalamus itself, together with its nervous connexions. Prof. Bargmann follows the scheme of the hypothalamic nuclei suggested by Spatz, and he refers by the term 'neuro-secretory pathway' to the nerve tracts which connect the n. supraopticus and n. paraventricularis above to the neural process of the neurohypophysis below. The debated question of the occurrence of nerve fibres in the adenohypophysis is touched on (the bulk of the evidence cited favours the view that such nerves exist), and the anatomy of the pituitary-portal vessels is then described.

The second and greater part of the monograph discusses the functional inter-relationships of the hypothalamus and hypophysis. The evidence that the former is the source of the hormones which help to regulate water and salt metabolism, blood pressure and uterine motility, as well as the contractility of certain myoepithelial elements in the mammary gland, is dealt with in detail. On this view the neural process of the neurohypophysis is merely a storage organ, the whole system, according to Prof. Bargmann, being akin to the type of hormonal complex found in invertebrates, with a group of cells producing the hormone, and a pathway by which it is transported to a storage-organ. More recent evidence that the neural process may exercise a less passive function is not considered.

The section dealing with the hypothalamico-adenohypophysial inter-relationship is less decisive than the one which deals with the neurohypophysis. This is not surprising, in view of the sketchy and speculative nature of most of the observations which have been brought to bear on the topic. Since the publication of Prof. Bargmann's monograph, there is published proof that the adenohypophysis can function normally when it has no direct contact with the hypothalamus.

Prof. Bargmann's monograph deals with a very active field of research. It was published in 1954, and in certain sections is, not surprisingly, already out-of-date. What Prof. Bargmann has written is, however, necessary reading for all new students of the subject; and before long he will no doubt provide an even more up-to-date account.

S. ZUCKERMAN

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1955

The Annual General Meeting of the Society for the Session 1955-6 was held on Friday, 25 November 1955 in the Department of Anatomy, Middlesex Hospital Medical School, London, W. 1. The President (Prof. W. J. HAMILTON) was in the Chair for the morning session. The communications in the afternoon were arranged in two sections with the newly elected President (Prof. R. D. LOCKHART) and Vice-Presidents (Prof. R. E. M. BOWDEN and Prof. G. M. WYBURN) in the Chairs at the various sessions.

The following are the authors' abstracts of the papers presented.

The epithelium of the adult anal canal. By E. W. WALLS
and R. P. GOULD. *Middlesex Hospital Medical School, London*

Several zones are described in the epithelial lining of the anal canal. No dispute exists with regard to three of these, viz. the upper zone continuous with the rectal lining and like it composed of simple columnar epithelium, the zone between the anal valves and the inter-sphincteric interval lined by stratified squamous epithelium devoid of hairs and skin glands, and the terminal part of the canal lined by true skin.

However, the nature of the epithelium in the zone between the valves below and the rectal type epithelium above is described differently by various authors. What does not seem to have been appreciated is that the lining of this zone shows considerable variation both between subjects and between different sectors of the canal of the same subject. It may consist of stratified columnar epithelium amongst which patches of simple columnar or stratified squamous type epithelium can occur, or it may consist mainly of stratified squamous epithelium.

In the upper part of the canal the change-over from one type of epithelium to another may be either gradual or sudden, but frequently there is interlocking of different types. In the zone between the valves and the terminal true skin the occasional presence of sebaceous and mucous glands has been observed in the present study.

The nature and extent of the anal glands which open into or near the anal sinuses are also described.

Topography in two human cyclops fetuses. By P. H. S. SILVER.
Middlesex Hospital Medical School, London

The material upon which this communication is based consisted of a 5-month male and a 6-month female, both offsprings of the same parents. The face and jaws of the former were embedded in celloidin and cut in the coronal plane. The latter specimen, which had macerated *in utero*, was not examined histologically.

In both specimens a proboscis projected from the forehead above the partly fused eyes. The cerebral hemispheres were fused. The upper lip and palate were complete, but a feature of the palates was the presence of three ridges running longitudinally, one in the midline and one on each side of the midline. The gums in the female specimen appeared normal, but in the male there was a projection in the midline of the upper jaw which had the naked-eye appearance of an erupted incisor tooth. On section, however, this proved to be derived from the gum, but it contained a tooth follicle. The gubernaculum of this tooth was connected to the surface epithelium by two strands of cells, one on each side of the midline.

Emotional sweating and the suprarenal cortex. By P. C. B. MACKINNON.
Middlesex Hospital Medical School, London

Under basal conditions the active sweat glands in an 'emotional' area of the skin were counted in ten women during the menstrual cycle and in 200 women during pregnancy. They were found to be significantly fewer in the luteal part of the menstrual cycle and in the 1st and 3rd trimesters of pregnancy, and the conclusion was reached that increased suprarenal cortical activity had probably been responsible in each case.

In order to obtain direct evidence that suprarenal cortical changes occur in the luteal part of the cycle, specimens were collected from a series of forty-seven successive post-mortems carried out in one London mortuary on women who died suddenly during reproductive life. Endometrial sections showed that forty-five of these had taken place in the luteal phase and only two in the follicular phase of the cycle.

This most remarkable finding was discussed, but its true significance will only emerge when a sufficiency of suprarenals in the follicular phase is available for comparison.

Contour reconstruction of the skull: a suggested new method. By D. I. G. BUNN
 and P. TURNER. *Middlesex Hospital Medical School, London* (introduced by
 E. W. WALLS)

The skull to be drawn is mounted on a universal joint inside a tank, and orientated in the desired plane. Water is then run into the tank until the skull is just submerged. The water level is then lowered in successive stages by $\frac{1}{4}$ – $\frac{1}{2}$ cm., depending upon the size of the skull, and at each stage a drawing is made of the outline produced by the water's edge meeting the skull surface. In this way complete maps are obtained of the skull held in the sagittal plane, Frankfurt plane, etc.

The drawing paper is mounted on the lens system of a camera placed vertically above the skull, and so arranged that it is maintained at a constant distance from the surface of the water. In this way both sharpness of focus and scale of enlargement are kept constant. Illumination is provided by strip lighting installed within the tank beneath the water. By excluding all other light the water surface acts as a mirror which reflects all light downwards, and so heightens the contrast between the brightly lit immersed portion of the skull and the poorly lit portion above the water.

From the contour maps obtained it is possible to gain a good 3-dimensional appreciation of the skull. Moreover, the maps provide a means whereby outlines can be prepared of any desired skull section without the need for actual cutting of the skull.

Certain disadvantages of the method inherent in the optical system employed were discussed.

Growth-rates in the foetal skull. By E. H. R. FORD.
St Thomas's Hospital Medical School, London

The growth of the foetal skull between 10 and 40 weeks has been studied by measurements on a series of seventy-six dissected foetal heads. During this time the forehead recedes and the occiput becomes more prominent. The anterior (prechordal) part of the cranial base has a growth-rate similar to that of the overall dimensions of the skull and brain, while the posterior (parachordal) part has a slower growth-rate. This differential growth is an example of the operation of the law of developmental direction, and results in flattening of the cranial base as a whole, with a reduction in angulation of 15–20° between the pre- and parachordal parts of the cranial base, and of 20–25° between the basiocciput and foramen magnum.

Most structures related to the chondrocranium, such as the foramina for the cranial nerves, and the internal ear, come to lie relatively nearer to the midline during growth, since more lateral growth takes place in the surrounding membrane-bones than in the

chondrocranium itself. When the otic capsule has ossified, however, it does not grow laterally from membrane but from growth cartilage remaining at its postero-lateral end.

In the face, width increases in relation to both height and depth; height increases in relation to depth, especially after 20 weeks, but there is no change in prognathism. The lower jaw lags behind the upper between 13 and 20 weeks owing to the late development of the growth cartilage of the mandibular condyle.

Effect of hindlimb amputation on the rat pelvis. By T. J. HARRISON.

Queen's University, Belfast

The hindlimb was amputated through the hip joint in twenty rats between 4 and 21 days after birth. They were killed between 17 and 234 days later and their pelves examined either macroscopically after removing the soft parts, usually after staining the bones with alizarin and clearing, or microscopically after sectioning and staining. It was found that asymmetry of the pelvis gradually developed following amputation. The pelvis was pushed over towards the amputated side caudal to the level of the sacro-iliac joints, so that the ilium became more curved than normal. There was no significant difference in the lengths of the ilium and ischium on the amputated as compared with the normal side. The acetabulum on the amputated side remained small, as Le Damany found to be the case in the rabbit (*Trav. Scient. Univ. de Rennes*, 1903). Histological examination showed that the acetabulum on the amputated side became filled with hypertrophied and fibrosed synovial membrane and fat. The centre for the os acetabuli appeared at the normal time.

The uptake of ^{35}S in cartilage homografts. By G. M. WYBURN
and P. BACSICH. *University of Glasgow*

Homografts of guinea-pig costal and xyphoid cartilage were transplanted for 3 weeks at which time the host animal was given an injection of ^{35}S . The grafts were removed 20 hr. after the injection and 5μ sections autoradiographed. The host's costal and xyphoid cartilage provided controls. Where the histological appearance of the grafts indicated healthy cartilage, with living cells and metachromasia of the ground substance, the autoradiographs resembled those of the controls, with dense packing of granules around the cells in the centre of the cartilage. No granules were present in necrotic areas, but surviving cells adjacent to such areas were mapped out by pericellular granular clusters. The evidence of persisting metabolic activity provided by the autoradiographs could, therefore, be correlated with the histological appearance, which presumably is a satisfactory criterion of the condition of the graft.

The experiment provided two interesting by-products. In the guinea-pig, the xyphoid cartilage continued to grow throughout adult life, whereas the costal cartilage only 'ticked over'; this difference in their respective activities was reflected in the variation of the granular density in the two autoradiographs.

Control autoradiographs of both xyphoid and costal cartilage showed differences from animal to animal, as though there were a 'metabolic cycle' such as suggested by Gersh and Catchpole for fibroblasts. The variations then would represent a phase difference in the cycle of the various animals.

Experimental bone induction by implants under kidney capsule of fracture callus, epiphyseal cartilage and other tissues in the rabbit. By J. B. BRIDGES and J. J. PRITCHARD. *Queen's University, Belfast*

Implantation under the kidney capsule in the rabbit of alcohol-killed autogenous or homogeneous (but not heterogeneous) fracture callus, provided it contained cartilage, resulted in bone or cartilage formation by the host tissues in almost every case. Similar results were obtained with alcohol-killed homogeneous epiphyseal growth cartilage.

Alcohol-killed bladder wall induced cartilage, but living bladder wall induced bone. No such induction followed the implantation of alcohol-killed adult periosteum, cortical bone, red bone marrow, skin, skeletal muscle or hyaline cartilage. Decalcified cortical bones were also unsuccessful.

Fracture callus retained its inductive capacity after immersion for 7 days in absolute alcohol or after heating to 55° C. for 10 min. Boiling, or treatment with 1 % NaOH, however, destroyed inductive capacity.

The induced bone began to appear 3-4 weeks after implantation on the walls of cavities eroded in the dead cartilage by invading blood vessels and connective tissue and proceeded after the manner of endochondral ossification in normal growth, to replace the cartilage by an ossicle of bone containing normal erythropoietic marrow. After 7-8 weeks this ossicle began to undergo osteoclastic resorption. Induced cartilage was less common, but when it appeared gradually underwent endochondral bone replacement also.

It was concluded that, in the rabbit, hypertrophic cartilage and bladder epithelium contained an inductor capable of converting ordinary connective tissue cells into osteoblasts and chondroblasts.

Microradiology of the blood supply of long bones. By M. BROOKES
and R. G. HARRISON. *University of Liverpool*

Following intravascular injection of 'Micropaque' (Damancy and Co.) microradiography of the femur and tibiofibula of the rat and rabbit demonstrates that the arterial supply and venous drainage show pronounced differences, especially in the rabbit, in which the paucity of the arterial supply contrasts with the profuse venous drainage.

The principal nutrient artery divides into ascending and descending limbs whose scanty branches show numerous tortuosities. Few arteries are seen in the epiphyses or metaphyses, and none in the cortex.

A central venous sinus drains profuse transversely disposed hair-like vessels. The ends of the bones show venous 'lakes'. The cortex of the bone is permeated by venous channels of a calibre large enough to admit 'Micropaque' particles. There is a rich vascular network along the endosteal surface of the cortex.

The impression gained is that a long bone is a predominantly venous structure. Arterial channels are few and largely confined to the medulla, whereas channels filled by retrograde injection through veins are greater in size and number, and many traverse the cortex. It is suggested that the latter are the so-called Volkmann's canals, which are therefore venous drainage routes.

Findings in the rat generally agree with the above, but show one important difference, the existence of a wide arteriovenous anastomotic bed in the medulla.

A rapid method of graphic reconstruction. By C. H. BARNETT.
St Thomas's Hospital Medical School, London

A technique is described by means of which perspective views of an object that has been serially sectioned may be drawn to scale. The apparatus used is a standard microprojector incorporating a cylindrical lens. Several views of the object can be produced in a few hours, giving information comparable with that derived from wax-plate reconstruction.

The blood supply of the optic nerve and chiasma. By E. J. STEELE (introduced by
R. E. M. BOWDEN) and M. J. BLUNT. *Royal Free Hospital School of Medicine and
St Bartholomew's Medical College, London*

Numerous observations have been made on the blood supply of the optic nerve and evidence conflicts on many points of detail. There is relatively little work on the supply of the optic chiasma and on the intrinsic vessels of both nerve and chiasma.

Present observations have been made on human material obtained from thirty-six post-mortems. In eighteen cases the vessels were injected with 'Neoprene' or 'Micropaque'. In seventeen cases sodium nitroprusside-benzidine staining was used and in one case serial sections were stained alternately with haematoxylin and Biebrich Scarlet and Mallory's triple stain.

Vessels reach the optic chiasma through a pial network which is supplied on the interior surface by the anterior superior hypophyseal, the internal carotid and posterior communicating arteries and on the superior surface by the anterior cerebral and anterior communicating arteries.

The arterial supply of the optic nerve is derived from branches of the anterior superior hypophyseal, the ophthalmic and posterior ciliary arteries and also from the central retinal artery.

In regard to the gross blood supply of the chiasma the findings are in general agreement with those of previous investigators. In the optic nerve there is unequivocal support for those who described intraneural branching of the central retinal artery, but there is no evidence of direct arterial anastomoses between branches of the central retinal artery and branches of the posterior ciliary arteries in the region of the lamina cribrosa.

Characteristic capillary patterns have been found in different parts of the chiasma and optic nerve.

The non-mamillary fibres in the post-commissural fornix.

By R. W. GUILLERY. *University College, London*

Degeneration studies of the fornix in the rat following hippocampal lesions of varying extent show that a large number of the post-commissural fibres do not reach the mamillary bodies. The majority of these non-mamillary fibres have the rostral third of the post-commissural fornix and pass dorsally into the antero-ventral and antero-medial thalamic nuclei. Some of the fibres pass through the ventro-medial parts of the reticular nucleus on their way to the antero-medial nucleus, while a few go to the nucleus rhomboideus, the nucleus reuniens and the contralateral antero-medial thalamic nucleus. None of the fibres from the post-commissural fornix reaches the antero-dorsal or parataenial nuclei. From the junction of the anterior and middle thirds of the post-commissural fornix a small group of fibres runs postero-dorsally and medially into the periventricular region of the postero-dorsal hypothalamus and rostral midbrain. The medial cortico-hypothalamic tract, passing from the anterior part of the post-commissural fornix into the periventricular region of the anterior hypothalamus, degenerates after lesions that include the fimbria. Some of these fibres pass postero-ventrally as far as the supra-chiasmatic nucleus, but the majority are lost at more rostral levels. A few fornix fibres by-pass the mamillary bodies to enter the mid-brain tegmentum through either the supra-mamillary region or the rostral end of the mamillary peduncle.

The non-mamillary fibres of the post-commissural fornix degenerate after lesions that spare the septum and the medial cortex. They most probably arise from the hippocampal formation itself.

The termination of the crossed tecto-spinal tract in the spinal cord of the cat.

By G. W. PEARCE (*Queen's College, Dundee*) and P. GLEES (*University of Oxford*)

In a previous communication the crossed tecto-spinal tract was shown to possess only a small spinal component since two-thirds of its fibres, as estimated by counts of degenerating fibres stained by the Marchi method, end in the brain stem.

Further experiments have been carried out to determine the precise site of termination of the crossed tecto-spinal tract in the grey matter of the spinal cord. The present series comprises twenty cats studied by the methods of Marchi, Glees, and Nauta-Gygax following lesions placed in the superior corpus quadrigeminum.

The Marchi method showed the tecto-spinal tract to be well defined from the first to the fourth cervical segments, but to be extremely small in the cervical enlargement.

The major part of the tract passed into the spinal accessory nucleus and this could be seen particularly well with the Nauta method as a region of degeneration, usually isolated and discrete, in the intermedio-lateral area. Both Nauta and Marchi preparations showed degenerating fibres passing dorso-laterally through the anterior horn towards the spinal accessory nucleus. This connexion was verified oscillographically by recording from the spinal accessory nerve in the neck.

At this level and in the cervical enlargement scattered degenerating fibres, few in number, could be seen in the intermediate zone, anterior commissural nucleus and in the dorsal half of the medial zone. Tecto-spinal fibres have been observed to pass dorsally through groups of anterior horn motor cells but not to end on them.

On the use of the Glees technique in the hypothalamus and preoptic regions.

By T. P. S. POWELL and W. M. COWAN. *University of Oxford*

Since bilaterally symmetrical terminal degeneration has been described in many of the nuclei of the preoptic and hypothalamic areas after lesions of such dissimilar structures as the frontal lobe, the amygdala and the hippocampus, a critical re-examination of the normal appearance of these areas in the Primate brain has been undertaken. Sections of these areas from three normal monkeys, one monkey with an extracranial lesion and a normal human brain, were stained according to the Glees technique. At all levels apparently typical terminal degeneration was seen in certain nuclei, especially the dorsomedial and ventromedial hypothalamic nuclei. As a comparison of this material with an experimental series of animals revealed no essential difference either in the site or severity of this 'degeneration', the significance of terminal degeneration in the hypothalamus in experimental material was discussed.

The size of the boutons terminaux in the sixth cervical segment of the spinal cord of the cat. By G. W. PEARCE. *Queen's College, Dundee*

Pearce & Glees (1954) classified the boutons terminaux of the third and sixth cervical segments of the spinal cord into five varieties: thin, thick, reticulated, fragmented and opaque. A comparison of their numbers in the postero-lateral (C6 only), antero-lateral, medial and intermedio-medial areas was made and their longest and shortest diameters were measured.

In a second series of six cats, similarly prepared and stained by the Glees (1946) method, the sixth cervical segment has been studied in detail as above not only to check and extend the results of the first series, but especially to gain additional data on the size of the boutons and their relative synaptic areas of the cells of the above four regions. Since it is clear that not all boutons are stained by the Glees technique no absolute values can be claimed. However, it is suggested that the technique is sufficiently repeatable to be of value as a method of comparing the relative numbers and size of boutons.

The following are the chief findings:

- (1) The results of the first series are confirmed.
- (2) There is a statistically significant difference in size between the reticulated endings on the motor and internuncial cells and between the thin endings on the motor and internuncial cells.
- (3) The synaptic area per cell section for reticulated endings is about one-third greater on the internuncial cells than on the motor cells.
- (4) The synaptic area per cell section for thin endings is one-third greater on C6 motor cells than on internuncial cells.
- (5) These results give additional support for the suggestion that there may be a difference in function or efficiency between the thin and reticulated endings.

The relationship between the cerebro-spinal fluid pressure and the occurrence of hydrocephalus in the young of female rabbits subjected to experimental hypovitaminosis A. By J. W. MILLEN and D. H. M. WOOLLAM. *University of Cambridge*

The occurrence of hydrocephalus due to maternal vitamin A deficiency has been described in new-born rabbits (Millen, Woollam & Lamming, *Lancet*, **265**, 1953). In order to study this syndrome, a colony of rabbits was established in 1954 and fifty-three litters have been born to dams subjected to experimental hypovitaminosis A. In this colony 163 young have been found to be hydrocephalic and fifty-two 'normal'. In sixteen of the 'normal' non-hydrocephalic rabbits the cerebro-spinal fluid pressure has been measured and found to be elevated up to 500 mm. of water (the average cerebro-spinal fluid pressure in rabbits is less than 100 mm. of water). The number of hydrocephalic young increases with the length of time the dam is on the diet before mating, and there is a greater percentage of hydrocephalic young in second litters of dams subjected to a continuous vitamin A deficiency.

The findings outlined above, supported by the similar experiments upon chicks subjected to a vitamin A deficiency (Woollam & Millen, 1955) indicate that an increased cerebro-spinal fluid pressure is an early sign of hypovitaminosis A, preceding any other sign of involvement of the central nervous system. The results of the experiments suggest that there is a close relationship between the finding of an increased cerebro-spinal fluid pressure in the young subjected to a moderate degree of deficiency and the occurrence of hydrocephalus in those suffering from more severe degrees of deficiency.

Iris reactions to foetal pineal homografts. By R. L. HOLMES. *University of Leeds*

Fourteen homografts of foetal pineal bodies (22-26 days) were transplanted into the anterior chamber of the eye of adult rabbits. The majority of these 'took' successfully. They showed considerable initial increase in size, with numerous mitotic figures in the pineal cells, and differentiation to the adult type of structure. Very large thin-walled blood vessels developed around the periphery of the graft, between it and the iris stroma, and the mass of pineal tissue developed a rich capillary bed.

Grafts examined at 40 days and later showed development of dense masses of cells in relation to the peripheral vessels. These masses consisted of small lymphocytes, some plasma cells, and foci of large (15μ) cells with pale-staining nuclei and pyronin-staining cytoplasm. Transitions between these and mature plasma cells were found.

Numbers of healthy pineal cells have been seen in grafts of four months' duration.

It is considered that these foetal grafts give rise to a mild but extended antigenic reaction. Development of a nodule of lymphoid tissue in relation to the graft occurs, within which the types of cells concerned in antigen-antibody reactions appear. It seems unlikely that differentiation and growth of the implanted tissue to adult form is accompanied by a corresponding increase in antigenicity.

Cytological studies of digital touch corpuscles. By N. CAUNA.
King's College, Newcastle upon Tyne

In the literature, Meissner's corpuscle is described as either a vesicle containing a semi-fluid ground substance or as a receptor organ of laminar, fibrous or cellular structure. The cells of the corpuscle have been variously described by different observers as rounded, spindle-shaped, unipolar or branching.

The present investigation is based on human material of 125 individuals classified according to age, sex and occupation. Cytological and nerve-staining methods, microdissection and various maceration techniques have been used.

It has been found that the corpuscle is entirely cellular apart from its elastic capsule. The cells are different in the superficial and deep parts of the receptor. The superficial part consists of multinucleated flattened circular cells of $2-4\mu$ in thickness and $30-40\mu$ in diameter. The nuclei usually occupy the periphery of the cells. They are large, oval with distinct nucleoli and lightly staining chromatin. Nuclear growth and division can be observed in these cells. The deep part of Meissner's corpuscle contains cells of irregular shape with small oval or rounded nuclei which do not show nucleoli and contain deeply staining chromatin similar to that of lemmocytes. The two types of cells in Meissner's corpuscle correspond to the two developmental elements of early stages.

Nerve endings are related to the corial surface of the superficial cells. Cells of the deep part of the receptor are not related to nerve endings. The spiral course of nerve fibres and the horizontal arrangement of endings are determined by the shape of the corpuscular cells.

Lymphocytes in thoracic duct lymph and bone marrow of the guinea-pig.

By V. B. PATHAK, W. O. REINHARDT and J. M. YOFFEY. *University of Bristol*

Following quantitative determinations of thoracic duct lymphocyte output in the guinea-pig, which indicated a Daily Replacement Factor of the order of 3.0, attention has been directed to the structure of the cells. These are almost exclusively small lymphocytes, though there are about 4 % of large cells. Though there is no question about the difference in size between the small and the large cells, lymphocytes of intermediate size, of which fortunately there are not many, present some difficulty from the point of view of measurement in smear preparations.

As seen in air-dried films stained with MacNeal's tetrachrome stain, no nucleolus is evident, though a 'chromocentre' may be seen in about 70 % of the cells. In wet-fixed films after digestion with deoxyribonuclease, the chromocentre does not completely disappear, but is smaller and rounder. Even after double digestion, first with deoxyribonuclease and then with ribonuclease, there is still present at the site of the chromocentre some residual substance which stains with iron haematoxylin, though it stains less intensely than before digestion with ribonuclease. Staining with methylene blue at pH 4.8-4.9, as suggested by Stockinger and Kellner, also brings out what appears to be a nucleolus both in air-dried and wet-fixed films. The same spherical structure may readily be stained by brilliant cresyl blue in supravital preparations.

In sections of bone marrow after Zenker fixation the appearance of the small lymphocyte is characterized by a well-developed nucleolus, exactly like the cells of thoracic duct lymph when similarly treated.

Observations on the development of the hepatic cell in the foetal sheep. By D. A. T. DICK. *University of Glasgow*

In an attempt to throw light on the functional development of the hepatic cell in the foetal sheep, measurements were made of the nuclear cytoplasmic, and total cell volumes and the nucleo-cytoplasmic ratio. Preparations were also made by the periodic acid-Schiff method to demonstrate glycogen, by the methyl green-pyronin method to show RNA, and by the sudan black method (using frozen sections) to show lipids. Staining techniques were standardized to provide preparations capable of crude quantitative comparison. Although preliminary observations provide no statistically significant evidence of changes in the volume of the nucleus or cytoplasm between the 29 mm. stage and term, there is nevertheless a suggestion that the nuclear volume may decline slightly and the cytoplasmic volume increase, and this is supported by the fact that there is a statistically significant increase in the nucleo-cytoplasmic ratio over the same period. Results on a 22 and 26 mm. embryo further suggest that prior to the 29 mm. stage the cytoplasmic volume and thus the nucleo-cytoplasmic ratio are increasing rapidly. Glycogen, estimated as diastase-labile PAS positive material, was found to appear first in scattered cells at the 29 mm. stage and thereafter to increase in amount until term. The cytoplasmic RNA concentration, estimated as

pyroninophilia extractable by 10 % perchloric acid in 16 hr. at 4° C., underwent a small but distinct diminution with increasing age. RNA was invariably uniformly distributed in the cytoplasm. No sudanophilia was detectable until the 345 mm. stage, and in later embryos increased amounts were seen. Sudanophilia was diffuse and uniform throughout the cytoplasm. The interpretation of these findings as evidence of functional development in the individual hepatic cell was discussed.

Some electron microscope observations on ultra-thin sections of hairs and hair follicles. By G. E. ROGERS (introduced by A. F. W. HUGHES). *University of Cambridge*

Hair follicles of the human and newly born mouse and hairs of the rabbit and sheep have been studied by the ultra-thin sectioning technique and utilizing a Siemens 'Elmiskop' electron microscope. By these means submicroscopic structural features of osmic-fixed material have been noted in the developing cells of the cortex and cuticle and the dendritic melanocytes.

Microfibrils of keratin (protofibrils) less than 100 Å in diameter and small mitochondria have been observed in the cytoplasm of presumptive cortical cells. The changes they undergo at different levels in the follicle have been followed. In contrast to the basically fibrillar nature of the cortex, the cuticle keratin has a granular origin and the development and location of this component has also been traced during fibre growth.

The cytoplasm of the pigment-producing melanocytes is again different from that of the presumptive hair cells. Numerous small mitochondria are found in addition to endoplasmic reticulum and melanin granules at different stages of formation. Mature melanin granules possess fine structure which is generally of a lamellated type.

It has been found possible to cut sections of intact hairs embedded in methacrylate sufficiently thin to reveal fine structure *in situ*. Previous knowledge of the fine structure of hairs has come from studies involving partial chemical breakdown to achieve electron penetration. Structural details such as cortical cell outlines and membranes, and the cuticle structure have been observed.

Some electron microscope appearances of the rat adrenal medulla.

By J. D. LEVER. *University of Cambridge*

With reference to their overall electron opacity, medullary cells are dark, light or intermediate in appearance. Dark cells contain abundant osmiophile adrenaline granules in a relatively opaque background cytoplasm. Light cells contain fewer secretory granules and are increased in number in cold-stressed animals. Great variation in appearance of adrenaline granules suggests their inception, within saccular investments, by an aggregation of micro-granules. In the denervated medullary cell these aggregate forms are rare, and a polymorphism of granules, many of which have halos of less osmiophile material, may suggest granule dissolution. A subendothelial space, often containing semi-opaque material, separates parenchymal and (blood sinusoid) endothelial plasma membranes; it has inter-parenchymal cell extensions. The appearance of nerve fibres and synapses is described before and after denervation.

The development of the circulation in the spleen of the foetal rabbit.

By O. J. LEWIS. *St Thomas's Hospital Medical School, London*

The development of the splenic circulation has been studied in the spleens of rabbit embryos injected with Monastral Fast Blue BNVS paste, and these have been correlated with histological sections. The primordial circulation of the spleen is found to consist of a plexus of channels in the mesenchymal rudiment, walled only by the mesenchymal cells, and connected by arterial and venous branches with the splenic vessels at the hilus. Arterial vessels elaborate from this plexus by differentiation of the living mesenchymal cells into

endothelium, the process penetrating into the interior of the organ from the hilus. These arteries are continuous with the remainder of the plexus, whose channels are dilating up to form the primitive pulp spaces. Later veins and venous sinusoids develop from those primitive pulp spaces nearer the hilus, and the circulation, when fully developed, consists of arteries supplying pulp spaces which drain into sinusoids and thence to collecting veins. The arteries in the late embryo form loops and arcades from which the branches supplying the pulp arise; this ensures distribution of blood at equal pressures to different parts of the developing pulp. Thus, from the earliest stages the circulation is 'open', but parts of the circulation later develop definite endothelial walls, though the basic open structure is unaltered.

The results of resecting the anal mucosa in mice. By R. J. O'CONNOR.
Westminster School of Medicine, London

The mucosa was exposed by suitable retraction at the anal verge, and areas 2–3 mm. square were resected. The muscularis mucosae was removed with the mucosa, but the main muscle layers were left intact. Such lesions progressively diminished in size and after 6–7 weeks only a small residual ulcer remained, or the site of the lesion was indicated by irregularities in the mucosal folds. Sections showed that the formation of new mucosa did not contribute to the closure of the lesion, which was due to the approximation of the edges. The muscularis mucosa retracted a considerable distance from the point at which the edges of the lesions came together, and the approximation took place equally well when the main muscle coats were removed from an area corresponding to the resected mucosa. The closure was therefore attributed to the fibrous tissue that forms as a result of the resection.

Some aspects of the problem of vaginal development.
By D. BULMER. *University of Aberdeen*

Investigation of a foetal series has indicated that the epithelial lining of the entire human vagina is derived from the cells of the urogenital sinus. In a 65 mm. foetus the sinus shows three distinct proliferations: one dorso-laterally, on each side, in relation with the openings of the Wolffian ducts, and one central proliferation from the dorsal sinus wall between the Wolffian openings. These three components fuse to form a single cellular mass, which, by the 140 mm. stage, extends throughout the entire length of the vagina.

While the sinus epithelium is extending cranially, the cells lining the lower part of the Mullerian utero-vaginal canal form a stratified squamous epithelium, which, in the 140 mm. foetus, lines the lower portion of the cervical canal. It is interesting to compare this with the gradual stratification of the Mullerian epithelium of the upper vaginal segment in such forms as the sheep. In the latter case, however, it seems likely that the stratified Mullerian epithelium persists as the vaginal lining, and is not displaced by an upgrowth of sinus epithelium as in the human foetus.

The stratification of the Mullerian epithelium is also significant in relation to Zuckerman's hypothesis (*Biol. Rev.* 15, 1940) of the different responses to oestrogenic stimulation of Mullerian and sinus derivatives. In the 140 mm. foetus the stratified Mullerian epithelium appears to coincide with the presence of oestrogenic hormone, indicated by a massive cornification of the lower end of the vagina.

Premeiotic mitosis: a new type of cell-division in mammals. By P. V. TOBIAS.
University of Witwatersrand, South Africa, and University of Cambridge

The nature of 'crust-like' nuclei and of compact mitotic figures sometimes occurring in mammalian seminiferous tubules has hitherto eluded satisfactory explanation. Cytological and cytogenetical studies on the gerbil's testis have thrown light on these two problems (Tobias, *Chromosomes, Sex-cells and Evolution in a Mammal*. London, Lund Humphries, in the press).

Crust-like nuclei occur regularly at a particular stage in the spermatogenetic wave cycle; they characterize spermatogonia shortly before the onset of meiosis and are not the result of somatic mutations as some aver. Such nuclei are in a partially prophase condition and, when they divide, the chromosomes—already partly condensed—contract excessively, resulting in the compact figures of premeiotic mitoses. The premeiotic mitosis is recognized as a new type of cell-division intermediate in character as well as in sequence between ordinary mitosis and meiosis. It occurs in mitotically dividing spermatogonia which are coming under 'meiogenic' stimuli.

In each spermatogenetic wave cycle, three successive premitotic mitoses quadruple the number of spermatogonia with dust-like nuclei. Then a non-mitotic change transforms most of these dust-like into crust-like nuclei, a change tantamount to that of early mitotic prophase. Each of the new crust-like nuclei undergoes two successive premeiotic mitoses, the first of which doubles the number of spermatogonia with such nuclei. The second doubles the number, but this time the daughter-cells have very small crust-like nuclei which undergo no further mitosis: they are the nuclei of preleptotene primary spermatocytes.

The crust-like nuclei and the compact mitoses thus bridge the important gap between mitotically dividing spermatogonia and meiotically dividing spermatocytes.

The organ of Jacobson. By V. E. NEGUS. *The Ferens
Institute of Otolaryngology, London*

Transparent reconstruction models have enabled the organ to be studied in detail. The communication deals with the size of the organ, the diameter of its ducts, and the various types of communication with the nasal fossa or mouth.

Suggestions are advanced as to the function, a question which presents considerable difficulties.

**An experimental study of the upper cervical course of the aortic nerve
of the horse.** By A. S. KING. *University of Bristol*

All previous investigators agreed that in its cervical course the aortic nerve of the horse was either fully independent as in the rabbit and a few other mammals, or semi-independent as in many wild and domestic Carnivora and Artiodactyla. In its semi-independent form it was said to lie inside the common vago-sympathetic connective tissue sheath as a discrete nerve, which rostrally became completely free of the sheath for a few centimetres to join the root of the cranial laryngeal nerve. However, these earlier investigators relied almost solely on direct anatomical observation and none examined more than five specimens.

The upper cervical course of the aortic nerve fibres has been investigated in experiments on twelve horses under chloral hydrate anaesthesia. The course of the fibres was traced by stimulating them electrically with an induction coil; reductions in arterial pressure and pulse rate, and hyperventilation, were taken as criteria for their presence.

There was no evidence for either a fully- or semi-independent aortic nerve. The aortic nerve fibres in fact were either diffusely scattered throughout the vagus or distributed more or less equally between the vagus and the plexus which occurs at the origin of the cranial laryngeal nerve. These findings were discussed.

The ictidosaur: a link between reptiles and mammals. By A. W. CROMPTON
(*National Museum, Bloemfontein, South Africa*) and A. D'A. BELLAIRS (*St Mary's
Hospital Medical School, London*)

In 1929 Dr Robert Broom (*Proc. Linn. Soc. N.S.W.* 54, 688) briefly described the remains of two small mammal-like reptiles from late Triassic Cave Sandstone deposits at Ladybrand in South Africa. Broom recognized these specimens as the most advanced of the known South African mammal-like reptiles, and placed them in a new order, the Ictidosauria. Despite their importance, however, the ictidosaurians have not previously been

described in detail and have not yet been given generic or specific names. Restudy of this material, and of remains of a third individual from the same source, by Dr A. W. Crompton has revealed further features of interest, among which are:

(1) The presence of both the reptilian (quadrato-articular) and the mammalian (squamoso-dentary) jaw articulations existing side by side. This feature is not known in other mammal-like reptiles. Angular, pre-articular and coronoid bones can be identified.

(2) The participation of the frontal bones in the side wall of the brain-case, as in mammals.

(3) The presence of small parasphenoid wings at the back of the skull base and of a vomer in the usual mammalian position. This finding supports the view of Parrington and Westoll (*Phil. Trans. B*, **230**, 305, 1940) that the mammalian and reptilian vomers are homologous, and that the former has not been derived from the parasphenoid.

(4) The extreme degree of reduction of the lumbar ribs, which is comparable with the mammalian condition.

Although the icetosaurs, like other advanced mammal-like reptiles, show a mosaic of reptilian and mammalian characters, they approach the mammalian organization very closely in many respects, and may represent an actual stage in the evolution of the mammalian from the reptilian class.

A new theory of the evolution of the mammalian ear-drum and tympanic cavity. By C. C. D. SHUTE. *University of Cambridge*

Current theories of the evolution of the mammalian middle ear fail to explain such features as the course of the chorda tympani between the pars tensa and Shrapnell's membrane, the supra-tympanic position of the tensor tympani and the forward position of the Eustachian tube.

A ventro-lateral diverticulum of the pharynx is described in lizards, probably associated functionally with pharyngeal respiration, which closely resembles the tubo-tympanic recess of mammals in its relation to the lower jaw. This structure, to which the name 'sulcus submandibularis' is given, may have been present in primitive synapsid reptiles, in close relation to the angulare when that bone developed a reflected lamina. When the masticatory muscles moved forward on to the dentary, and pharyngeal respiration was abandoned, the wall of the sulcus submandibularis could have been converted into the inner layer of a functional tympanic membrane, by coming into contact with an extension of the external auditory meatus. After the loss of the depressor mandibulae, the sulcus submandibularis would no longer have been separated from the tympanic cavity, and the Eustachian orifice could have been brought to its mammalian position in the nasopharynx by forward growth of the cochlea.

Shrapnell's membrane is regarded as a new formation in mammals bounding the attic region, which is required to accommodate the bodies of the malleus and incus. The posterior malleolar fold marks the original roof of the tympanic cavity. The cartilages of Paauw and Spence are discussed. Gaupp's identification of the mammalian goniale with the reptilian pre-articulare is upheld.

Bone repair in the salamander. By J. BOWDEN and J. J. PRITCHARD. *Queen's University, Belfast*

In mammals and reptiles bony union after fracture of a long bone is generally preceded by cartilaginous union, the cartilage initially formed being rapidly replaced by endochondral ossification as in normal growth at the metaphysis. In the frog it has been shown that repair of a limb bone proceeds to the stage of cartilage union after which endochondral ossification occurs very slowly and incompletely so that bony union is indefinitely delayed.

It has now been shown from a study of fracture repair in the humerus of the salamander that while cartilage union has occurred in about 6 weeks, endochondral ossification has not begun after 6 months. Moreover, the cartilage remains uncalcified.

It is suggested that failure of endochondral ossification in amphibian bone repair is a reflexion of a generalized reduction in ability to replace cartilage by bone in these animals which is otherwise shown by the retention of cartilage in much of the normal adult skeleton. In the frog, but not in the salamander, endochondral ossification does not occur during the normal development of the appendicular skeleton.

Experimental alteration of cranial suture patterns. By F. G. GIRGIS
and J. J. PRITCHARD. *Queen's University, Belfast*

In a previous communication the authors showed that damage to the cranial vault of rats *in utero* led to abnormal suture patterns during subsequent growth. It was anticipated, in view of Troitsky's (1932) findings, that damage to the cranial vault after birth, when the bones had met and become united by sutural fibrous tissue, would not result in alterations to the sutural pattern and that individual bones would not extend into the territories of their neighbours. However, it was found that damage produced by cautery to the growing margin of the parietal bone in neo-natal rats always resulted in overgrowth of the undamaged bones with consequent departure from the normal sutural pattern.

It was concluded that Troitsky was wrong in assuming that the sutural connective tissue prevents bones extending their territory at the expense of a neighbour. Rather it would appear that sutures are free to move during the growing period and that the direction of movement depends on the relative growth potentials of the edges of the bones involved. Furthermore, it would appear unlikely that the initial position of the sutures is predetermined except in so far as the position of ossification centres and rates of spread of osteogenesis from these centres is predetermined.

Cartilage in repair of the skull vault. By F. G. GIRGIS
and J. J. PRITCHARD. *Queen's University, Belfast*

Cartilage is rarely found during the repair of simple fractures of the skull vault. Three explanations for this have been put forward, viz.: (1) that the skull vault, having developed in membrane, lacks the ability to form cartilage, (2) that pressure and shearing stresses across the fracture line must be present if cartilage is to form, and that these are not found in repair of the skull vault because of the rigidity of the skull, and (3) that cartilage is a response to ischaemia at the fracture site and that under ordinary circumstances the blood supply to the skull is more than adequate for bone formation during repair.

It was decided to test the third hypothesis experimentally. Multiple cuts were made in the skull vault of thirty-five neo-natal rats in such a way that the blood supply to at least part of the fractured bone must have been seriously reduced. In the majority of cases the pericranium was scraped from the skull also. Cartilage was found on histological examination in seven of the thirty-five skulls, and in three of these cartilage union was present. On analysis it was determined that cartilage was only present between 6 and 17 days after fracture and was most common around the tenth day. Moreover, cartilage was confined to those specimens in which the pericranium had been scraped at the time of fracture.

It was concluded that ischaemia of the fracture site promotes the formation of cartilage during repair.

FEBRUARY 1956

An ordinary Meeting of the Society for the Session 1955-6 was held on Friday, 24 February 1956 in the Department of Anatomy, London Hospital Medical College, Turner Street, London, E. 1. The President (Prof. R. D. LOCKHART) was in the Chair.

The following are the authors' abstracts of the papers presented.

Delayed implantation in the badger (*Meles meles* L.). By R. J. HARRISON, E. G. NEAL and C. J. TURNER. *London Hospital Medical School*

Previous accounts of reproduction in the badger were given by Fischer (1931) for Germany, by Notini (1948) for Sweden and by Neal (1948) for south-west England. Fischer estimated that there was a period of delayed implantation of 4 months. Notini suggested 8 months and on the basis of Neal's observations a delay of from 2 to 8 months can occur. None of these workers had, however, made exhaustive observations on the ovaries. For this report the ovaries of forty-five adult badgers, obtained at different times of the year were serially sectioned. During the period March-June the average number of blastocysts found in each animal was 2.3 (range 1-3), the average number of corpora lutea was 4.0 (range 2-7). From August to December the average number of blastocysts in each animal was 3.0 (range 2-5), that of the corpora lutea increased to 6.2 (range 2-11) in each animal. After implantation (January) the average number of embryos was 3.1 (range 1-4) and the corpora averaged 6.5 (range 2-11) in each animal. Evidence of ovulation was found in animals with unimplanted blastocysts already in the uterus, thus accounting for the increase in corpora lutea. Examination of the vaginal epithelium suggested there may be from one to three cycles during delay, yet the uterine mucosa showed little evidence of activity. There was little evidence of addition or replacement of blastocysts. A slight increase (5-10%) occurred in the maximum diameter of the zona pellucida during delay. A large perivitelline space was consistently present, as noted by Notini, but little change was detected in the characteristics of the blastocysts during delay.

Delayed implantation in the southern elephant seal (*Mirounga leonina*). By W. NIGEL BONNER (introduced by R. J. HARRISON). *Falkland Islands Dependencies Survey and London Hospital Medical College*

In the southern elephant seal there is a delay in implantation of about 4 months. Three specimens have been obtained during the period of delay in two of which the blastocyst has been recovered. Five specimens covering the first 4 months of growth of the foetus have been examined.

During the period of delay the blastocyst lies free in the lumen of the uterus. There is apparently a considerable increase in size as Gibbney (1953) records a 0.3 mm. blastocyst from mid-December, while a 1.6 mm. blastocyst was recovered in late February and a 2.6 mm. blastocyst in early March. It is possible that this specimen was on the point of implanting.

The youngest corpus luteum from an animal containing a foetus shows a great increase in vacuolation of the luteal cells and extensive vascularization of the gland compared with specimens with a blastocyst. A decrease in lipid in the luteal cells occurs in the early growth of the foetus, but the lipid content increases later in pregnancy.

The specimen containing the oldest blastocyst shows an increase in the height of the uterine epithelium, from 10μ to $20-40\mu$. There is little change in the state of the uterine glands. The vaginal epithelium of this specimen is more complexly folded and a concentration of alkaline phosphatase occurs in the epithelium and in the vaginal secretions. Disulphide groups also occur in the secretion at this stage. These changes are reversed in the specimen containing the youngest foetus, but similar conditions are observed later in pregnancy.

Extra-dural vertebral venous systems in mammals. By J. W. D. TOMLINSON.
London Hospital Medical College

The findings of a large intravertebral extra-dural vein, situated dorsal to the spinal cord in Pinnipedia stimulated an investigation into the pattern and functions of the vertebral venous systems as present in other mammals, including man. The importance of this system in man, particularly with regard to the metastasis of prostatic carcinoma to the vertebral column, was emphasized by Batson (1940).

The system has been examined by dissection, by making Marco resin casts, and by experimental procedures in a variety of mammals. In the majority this venous system consists of two longitudinal venous sinuses which have very thin walls and no valves, lying ventral to the spinal cord, outside the dura mater, and which communicate with each other, with the veins of the spinal cord, with a venous plexus in the paravertebral muscles, with the azygos venous system (Bowsher, 1954) and with the intracranial venous sinuses. Modifications of this pattern are found in several mammals, particularly in Pinnepedia, Cetacea and Edentata, and are described and discussed. Injection of radio-opaque substances into the extra-dural veins in living Pinnipedia indicates the free communication of the system with the abdominal and cranial veins.

Experiments have been carried out on dogs, using radio-active NaCl as a tracer, to determine the effect of ligation of certain vessels, including the inferior vena cava and azygos vein, on the flow from hindlimb veins to the superior sagittal sinus. In nearly all the experiments the injected isotope reached this cranial sinus very rapidly.

Histological evidence for the innervation of human dentine.
By R. W. FEARNHEAD. *London Hospital Medical College*

Many attempts have been made to establish histological evidence for the extreme sensitivity of human dentine. Whether nerve fibrils in the dentine are situated in the dentine matrix between the dentinal tubules, or within the tubules, is, however, still a matter of some controversy. Many of the attempts to show nerve fibres in dentine are open to criticism on the grounds that insufficient care was taken to exclude by histological methods sources of artefact which could lead to misinterpretation of silver impregnated structures in dentine. In this study decalcified sections of human molars and premolars from individuals between 10 and 24 years of age have been impregnated with silver by a modified Holmes technique. Very small beaded nerve fibrils are found within the dentinal tubules between the tubule wall and the odontoblast process. Odontoblast processes, and the matrix of the 'translucent' zone around the dentinal tubules have been demonstrated by Held's molybdic acid-haematoxylin stain and unstained sections have been also studied by means of the phase-contrast microscope. An attempt is made to exclude the possibility of confusing these fibrils with (a) silver impregnated reticulin fibres, (b) dentine matrix, (c) odontoblast process or (d) precipitated silver trapped between odontoblast process and tubule wall. Ultra-thin sections of undecalcified dentine fixed in buffered pH 7.2 osmium tetroxide and embedded in methyl-methacrylate were cut with a diamond knife and examined with the electron microscope.

The formation of the hair germ in hair follicles. By WILLIAM MONTAGNA
(introduced by R. J. HARRISON). *Brown University, U.S.A.*

There is abundant evidence against the view that hair follicles during the transition from the active to the quiescent state set aside a special 'hair germ'. Our observations on the effect of X-rays on human hair follicles are of particular interest in this respect. After the scalp is X-irradiated with 600-800 r. the entire matrix of the bulb degenerates. The upper bulb degenerates more rapidly and becomes reduced to an attenuated core of cells. Since a club fails to form, the hair is shed and the cells of the outer sheath close the gap, reducing

the follicle to a solid cord. The lower part gradually degenerates, the cord becomes shortened and its base retreats within the dermis. The dermal papilla, always in contact with the base of the cord, retreats upward with it. After 5–7 weeks the base of the cord and its associated papilla rest halfway up in the dermis. Since the lower half of the hair follicle has degenerated, the definitive cord is composed entirely of cells from the outer sheath in the upper half of the follicle. The cells of the cord, and particularly those cells in contact with the dermal papilla, burst into mitotic activity. The cord grows in length and cells grow around the dermal papilla, forming a new bulb. This is the onset of recovery and growth of a new hair follicle. The lower half of the hair follicle, including the bulb, is a transient structure which is formed anew from the cells of the outer sheath at the onset of each hair generation. The outer sheath in the upper half of the follicle must be the source of the 'hair germ'. During the periods of growth and rest, the outer sheath in the upper half of the follicle is the only permanent portion of a hair follicle. These events are probably similar to those which occur normally during cycles of growth in each follicle.

Some histochemical observations on purkinje cells. By G. H. BOURNE.

London Hospital Medical College

Purkinje cells give a positive response to a variety of histochemical techniques. However, cells which have virtually no activity can be seen alongside others which give a strong positive reaction. There is some doubt as to whether this represents cyclical activity in the Purkinje cells or is due, at least in older animals, to the first stage of degeneration of these cells which precedes their progressive disappearance in animals over middle age. This problem is discussed.

A variety of very active phosphatases can be seen in the prominent nucleoli of Purkinje cells; since nucleolar activity has been correlated by some workers with protein synthesis, it may indicate considerable activity in this respect by these cells.

Some observations on the histochemistry of goat foetal cardiac and skeletal muscle.

By EVELYN B. BECKETT and G. H. BOURNE. *London Hospital Medical College*

Cardiac (ventricular) muscle and four skeletal muscles were studied from each of a series of goat foetuses of from 2 to 18 in. C.R. length.

A cholinesterase technique was carried out on frozen sections of skeletal muscle and succinic dehydrogenase technique on frozen sections of both skeletal and cardiac muscle. Acid and alkaline phosphatases and adenosine-5-monophosphatase preparations were made from acetone-fixed specimens of both types of muscle.

Cholinesterase was present at an early stage. It increased in activity with foetal length. Structural differentiation of end-plates could be followed in these preparations.

Skeletal muscle showed a light to moderate reaction and cardiac muscle a moderate to strong reaction for succinic dehydrogenase activity. This increased with age, but did not reach adult levels of intensity.

Both cardiac and skeletal muscle showed great variations in content of all three phosphatases. In skeletal muscle, intensity could not be correlated with foetal age, but in cardiac muscle there seemed to be a slight tendency for alkaline phosphatase and adenosine-5-monophosphatase to increase as foetal length increased. The reaction intensity of these phosphatases in foetal muscle was in each case compared with that in the adult tissues.

Autoradiographic studies of uptake of ^{35}S in normal and scorbutic guinea-pig tissues. By C. RUTH HILL and G. H. BOURNE. *London Hospital Medical College*

Autoradiographic techniques were used in these studies to try to confirm certain biochemical findings by previous workers, and in particular to discover the sites of variation in uptake of ^{35}S -labelled Na_2SO_4 by scorbutic and normal guinea-pig tissues.

Pair-fed guinea-pigs of 200–250 g. were fed on rat cake supplemented with vitamins A and D, and in the case of control animals were given an intraperitoneal injection of 15 μ c./g. body weight of ^{35}S -labelled Na_2SO_4 in 0.9 % saline with 0.02 % Na_2SO_4 as carrier. They were killed about 4 hr. after injection, tissues were removed, fixed and embedded and contact and stripping-film autoradiographs were prepared.

Previous workers have shown by biochemical methods that ^{35}S -labelled Na_2SO_4 , when injected into rats, is incorporated chiefly into the chondroitin sulphate of cartilage, and that the uptake by chondroitin sulphate of scorbutic costal cartilage is only about one-third that of normal costal cartilage. Autoradiographs of costochondral junctions, digit, knee joint, nasal cartilages, lung and trachea, show that in scorbutic cartilage there is either a decreased uptake of ^{35}S , or the activity, though strong, is confined to the cartilage cells, in contrast to the normal cartilage where there is activity in both cells and ground substance. Since in scurvy the synthesis of ground-substance material by certain cells is believed to be inhibited, and abnormal products may be formed which cannot be concerted into the necessary intercellular material, this observation is of special interest. So far no significant variations in uptake of ^{35}S have been observed in the many soft tissues examined.

Sulphydryl and disulphide groups in the human vaginal epithelium. By A. W. ASSCHER and C. J. TURNER (*London Hospital Medical College*) and C. H. DE BOER (*The Women's Hospital, Liverpool*)

Sulphydryl and disulphide groups in sections of human vaginal epithelium were studied with the method of Barnett and Seligman in twenty-eight biopsy specimens taken from different subjects at different stages of the menstrual cycle. The findings were:

(1) *Sulphydryl groups.*

The deepest six to ten layers of the epithelium showed positivity of the cellular cytoplasm and intercellular bridges; the nuclei in this zone were negative. The superficial portion of the epithelium displayed marked positivity of the cell walls; some of the pyknotic nuclei of this zone also reacted positively. During the follicular phase of the cycle an intermediate zone could frequently be distinguished; it showed more intense —SH group positivity of the cell walls than the overlying superficial cells. This zone appeared to broaden during the follicular phase and approached the surface towards the middle of the cycle. During the luteal phase this zone narrowed and finally disappeared due to desquamation.

(2) *Disulphide groups.*

These were demonstrable in the walls and peripheral cytoplasm of the cells in the intermediate zone. Their greatest concentration (as judged by the colour intensity of the reaction) was attained in specimens taken between the 13th and 17th days of the cycle.

These findings were compared with the distribution of vaginal —SH and —SS groups of the mouse (Asscher & Turner, 1953). The validity of equating certain colorations observed with Papanicolaou's and Gurr's vaginal smear stains with the occurrence of cornification was discussed.

The histochemical localization of alkaline phosphatase in transitional epithelium and its relationship to fatty material. By B. F. MARTIN. *University of Sheffield*

Sections of the bladder and ureter of four species (rabbit, guinea-pig, rat and mouse) were subjected to both the 'cobalt' and the 'azo-dye' techniques for alkaline phosphatase. The results with the two techniques were identical, except that diffusion of the reaction to nuclei occurred very readily with the 'cobalt' method. The lining epithelium of the bladder showed no reaction in the superficial cell layer, but deep to this the majority of the epithelial cells showed a strong reaction, localized to the apical parts of their cytoplasm, in the form of 'caps'. In the ureter, the reaction was chiefly in the middle layers of the epithelium and the cap-like reaction was particularly striking. The rabbit and guinea-pig showed also a strong

reaction in the narrow connective tissue band constituting the tunica propria; the rat and mouse did not.

An intracellular localization of alkaline phosphatase was less common than at specialized free surfaces of cells, but in some regions (e.g. lining epithelium of small intestine), a reaction was found in the Golgi zone. The possibility was envisaged that this might be the case with the cells of transitional epithelium. Application of the osmic acid technique for the demonstration of Golgi material showed that in the bladder and ureter osmiophil material was present as an apical cap-like accumulation in the lining epithelial cells, except those of the surface layer. The Sudan Black techniques gave an essentially similar result, whereas the epithelium was negative with Sudan IV. Silver techniques for the Golgi 'apparatus' gave less consistent results.

The marked similarity of the pictures obtained with the osmic acid, Sudan Black and alkaline phosphatase techniques indicated that in transitional epithelium, alkaline phosphatase was present in a specialized apical zone of the cells, where material of a fatty nature, related to Golgi material, was also accumulated.

Histochemical demonstration of alkaline and acid phosphatases in electron micrographs. By D. BRANDES (introduced by G. CAUSEY). *Royal College of Surgeons of England*

The Gomori techniques for acid and alkaline phosphatases were modified to apply them to material for electron microscopical observation. Small pieces of tissues were fixed in isotonic buffered 1 % osmium tetroxide for periods ranging from 5 to 30 min. They were then washed in Tyrode solution and in distilled water. For the alkaline phosphatase technique the material was incubated in Gomori's mixture for 1-2 hr. The precipitate of calcium phosphate was visualized by the von Kossa method. For acid phosphatase, the tissues were incubated in Gomori's mixture for $\frac{1}{2}$ -6 hr. and the precipitate visualized with ammonium sulphide. For demonstrating alkaline phosphatase in *Chlorella vulgaris*, the tissues were first incubated in the Gomori mixture for $\frac{1}{2}$ -2 hr. and then fixed in osmic acid vapour. In all cases controls were run by incubating in mixtures without substrate or by adding a suitable inhibitor.

In the mouse intestinal epithelium the reactions appeared in the brush border as a dense precipitate in relation to the parallel cylindrical projections from the surface of these cells. The exact relationship between the site of the histochemical reaction and the cell membrane was discussed.

The acid phosphatase test also showed an intense reaction in the juxta-nuclear region, towards the luminal border. Neither the large vacuoles nor the membranes of the Golgi apparatus seemed involved. In the algar material, it has been possible to demonstrate the reactions for alkaline phosphatase on the cell surface, giving visual confirmation of previous biochemical observations.

The absence of alkaline phosphatase reaction inside cells may be due to failure of the reagents to permeate intact cell membranes. This possibility is being investigated in prostatic cells.

Epithelialization of mucosal lesions in the gall bladder of the cat.

By R. M. H. McMINN and F. R. JOHNSON. *University of Sheffield*

The behaviour of the epithelium of the gall bladder in the cat has been studied during the healing of experimental mucosal lesions. An area of gall bladder wall approximately 0.5 cm.² in size has been denuded of its mucous membrane by scraping. Twenty-four animals have been used for these experiments and allowed to survive after operation for periods ranging from 18 hr. to 28 days. The site of the lesion has been removed for histological and histochemical examination.

It has been confirmed that mitotic activity in gall bladder epithelium is normally very low. Twenty-four hours after operation, however, many mitotic figures can be seen at the

wound margins and in the surrounding epithelium, and epithelial cells have begun to move over the floor of the lesion. Epithelialization is completed in 5 or 6 days, prior to which there appears to be some diminution in the intense mitotic activity. Mitosis in connective tissue cells in the floor of the lesion is also detectable within 26 hr. of operation.

These findings have compared with the reactions known to occur in other regenerating epithelia, and histochemical observations are also reported.

Bilateral fistulae of the nasopharynx in a living adult. By C. P. WILSON
(introduced by J. KIRK). *Middlesex Hospital Medical School, London*

An account is given of the fortuitous discovery of bilateral fistulae of the nasopharynx in a living adult. The orifices of the fistulae are situated in the nasopharynx just below and behind those of the Eustachian tubes and the tracks of the fistulae pass upwards and laterally for a distance of about 1 in., roughly parallel with the line of the Eustachian tubes. Photographs and X-rays are shown and the possible derivation and significance of the fistulous tracks discussed.

Congenital abnormalities of the cervical vertebrae. By SIR RUSSEL BRAIN, BT.
(introduced by R. J. HARRISON). *London Hospital Medical College*

A study of lesions of the cervical spinal cord has shown the clinical importance of congenital abnormalities in this region, ranging from primary basilar impression, the radiological criteria of which is discussed, through occipito-atlantal and atlanto-axial malformations, to abnormalities of fusion and defective separation of lower cervical vertebrae. Radiological examples of these are shown, and their relationship to congenital malformations of the nervous system discussed.

The problem of encapsulated nerve endings in the conjunctiva. By D. R. OPPENHEIMER, ELISABETH PALMER and A. G. M. WEDDELL. *University of Oxford*

A number of nineteenth and early twentieth century investigators have described various forms of organized, encapsulated nerve-endings irregularly distributed in the mammalian conjunctiva. Other workers during the same period have denied their existence. However, the standard text-books nearly all regard their occurrence as an established fact; and the presence of so-called 'end-bulbs' has been made the anatomical basis of a widely held theory that they are the specific receptors for the sensation of cold.

In the present investigation, over forty mammalian conjunctivae have been examined by various techniques. In the whole of this material, which includes various primates, a total of four encapsulated endings have been found in two specimens, both of senile human material. Otherwise, only simple, free terminations have been encountered.

The conclusion is drawn that encapsulated endings in the conjunctiva are the exception rather than the rule, and may well be pathological appearances; and that the theory associating end-bulbs with cold sensation should be treated with scepticism.

Early changes in the mouse spinal cord following irradiation with focused ultrasound. By DAVID BOWSER. *University of Liverpool*

The thoracic region of the spinal cords of mice were irradiated with focused ultrasound at 2.5 megacycles so as to produce monoplegia or paraplegia. They were killed at 1-24 hr. after the injection of trypan blue. The cords in the region of the lesion were very soft and friable, and showed staining with trypan blue and a little blood. Nerve cells in this region showed an early foamy swelling of the cytoplasm, sometimes with rupture of the cell membrane. With increasing time, these cells tended to disappear. The ependyma and meninges were undamaged, and haemorrhage was very slight, most of the bloodstaining being due to vascular engorgement. In the monoplegic animals, damage was more or less confined to one side of the cord, and areas above and below the lesions showed no damage.

The evolution of the parotid gland and its secretomotor innervation.By C. C. D. SHUTE. *University of Cambridge*

Evidence is put forward for a new interpretation of the parotid: namely that it is not a mammalian neomorph, but is derived from the glands on the outer side of the lower jaw in reptiles. The ultimate position of the opening of the parotid duct—on to the cheek or, in some mammals, the upper lip—is ascribed to growth changes in the region of the angle of the mouth. In all cases the mucous membrane around the duct orifice is supplied by the buccal nerve and the overlying skin by the auriculo-temporal nerve. The anlage of the parotid is formed from the mandibular process, whereas Chievitz' organ is an inclusion between the maxillary and mandibular processes. The otic ganglion is homologized with a motor ganglion on the mandibular ramus of the trigeminal nerve in reptiles. Preganglionic fibres are thought to reach this ganglion via the chorda tympani and an autonomic trunk accompanying the stapedial artery. In primitive mammals the lesser superficial petrosal nerve which receives a large contribution from the cranial sympathetic trunk is the nervus comitans of the stapedial artery, and the otic ganglion may get much of its preganglionic supply from the chorda tympani. The cranial autonomic pattern in mammals and reptiles is broadly similar, and some of the differences can be correlated with reduction of the stapedial artery and the release of the post-dentary membrane bones from the lower jaw. Jacobson's nerve in mammals is not the homologue of Jacobson's anastomosis in reptiles. The term 'parotid' should not be applied to true upper lip glands, such as poison glands in snakes.

New neolithic remains from West Kennet Long Barrow, Wiltshire.By L. H. WELLS. *University of Edinburgh*

The West Kennet Barrow was re-excavated in the summer of 1955 under the direction of Professor Stuart Piggott, and four burial chambers not observed by Thurnam in 1859 were discovered. From these the remains of at least twenty adults and ten infants and children were recovered. The burials had been considerably disturbed, apparently during later neolithic times; most of the crania and a number of long bones had been removed. There was no evidence to suggest the secondary burial of remains already skeletonized. The surviving remains showed many evidences of bony injury and disease, as well as developmental anomalies some of which may have been familial in nature. This material also permitted a reconsideration of the osteological features ascribed to neolithic man by Keith, Cameron and Cave.

The vascular arrangements within the human prostate gland.By E. J. CLEGG. *University of Liverpool*

Previous work on the intrinsic vasculature of the prostate has depended almost entirely upon the radiography of glands injected with various radiopaque media. In the present series use has been made of 200 μ transverse, sagittal, and coronal sections stained by Pickworth's method. The findings agree with most previous authors in that three distinct arterial zones can be observed, a capsular zone with tortuous vessels ramifying in the capsule and outer part of the gland, an intermediate zone, consisting of centripetally running vessels supplying the glandular acini and terminating by passing round the lateral angles of the urethra and supplying the urethral crest, and a central zone of vessels around the urethra itself. This urethral plexus is nowhere very rich, although it increases in extent towards the apex of the gland. Superiorly the vessels in the urethral crest are most numerous, but inferiorly a large plexus of vessels in the anterior wall of the urethra supplies the whole of that structure.

The two ejaculatory ducts carry with them a separate connective tissue sheath contain-

ing large numbers of vessels, both arteries and veins, which ramify in the lower part of the urethral crest.

Peripherally, the glandular acini receive large numbers of vessels from the capsular plexus, and centrally the relatively infrequent glandular elements are also well vascularized, but in the intermediate zone the acini are supplied by occasional vessels only.

Placentation in a full-term spotted hyena (*Crocuta crocuta* Eryxleben).

By W. R. M. MORTON. *Queen's University, Belfast*

A multiparous spotted hyena bitch which died during labour on 3 January last, was examined a few hours after death. The perineum was distended by the presenting head of a pup, the face of which could be palpated through the vaginal orifice. An episiotomy was performed and the foetal head exposed, but traction failed to deliver the pup, which was lying sagittally with the chin towards the symphysis pubis in the 'persistent occipito-posterior' position. The pubic arch had to be removed before the pup could be extracted. The abdomen was largely occupied by a greatly distended bicornuate uterus, which on opening was found to contain two other pups besides that obstructing the vaginal canal. The placentae of two of the pups had become detached during labour, but that of the third had remained in position over most of its extent lying slightly obliquely in the left horn. The placental zone formed a complete band of varying width, being widest (11 cm.) antimesometrially and narrowest (3.5 cm.) near the umbilical vessels. It varied in thickness from 1.4 cm. at the lower edge to 0.9 cm. at the upper edge, and was approximately 40 cm. circumferentially. The histological appearances of the placenta, and of the two ovaries, each of which contained corpora lutea, were described.

Ossification patterns in twin and triplet sheep foetuses.

By R. N. SMITH. *University of Bristol*

For this preliminary report fifty-five sets of twins and ten sets of triplets have been examined. When the ossification patterns of two siblings of the same sex and size (crown-rump length agreeing to within 10 mm.) are compared one often has more ossification centres than the other. If the pairs are of the same sex but different size and there is a difference in the number of the centres, it is usual for the larger foetus to have the greater number. When the size is the same, the sex opposite, and there is a difference in the number, then it is often the female which has more. In pairs of siblings of different size and opposite sex the larger foetus usually has the greater number of centres irrespective of the sex.

Oral smear determination of chromosomal sex.

By J. B. D. TORR
and A. D. DIXON. *University of Manchester*

A sex difference in the morphology of intermitotic nuclei has been known for some time, namely the presence in many of the cells of the female of a mass of chromatin in the vicinity of the nuclear membrane. More recently a method for the determination of chromosomal sex by the examination of smears of oral epithelium has been described.

In this investigation smears from 260 individuals were taken from the inner surface of the cheek, stained with cresyl violet and examined. The age range was from 16 months to 60 years and there was also some racial variation. It was found possible to forecast with complete accuracy the sex of the individual from the smear, provided that only cells fulfilling certain criteria were accepted for examination—those with large, circular and palely stained nuclei with no crenation of the nuclear membrane; no folding of the cell membrane or obscuring of the nucleus by adjacent cells or bacteria; and, finally, only darkly stained plano-convex bodies in contact with the nuclear membrane were considered to be sex chromatin.

In the majority of female smears 30% to 50% of cells with these characteristics were found to possess sex chromatin, whereas none was seen in most male smears.

The sex can be determined accurately from an examination of twenty-five cells of the type specified, irrespective of age or race.

Post-mortem morphology of chromosomal sex. By A. D. DIXON
and J. B. D. TORR. *University of Manchester*

The existence of a sex difference in nuclear morphology may be of importance in medico-legal cases, where it might be possible to determine the sex of an individual from small fragments of tissue. The problem of the persistence of sex chromatin (*a*) in dissecting-room material and (*b*) in the skin of full-term fetuses has been investigated.

Small portions of tissue were removed from seven dissected cadavers, their ages ranging from 60 to 78 years. Preservation procedures had been commenced 2–5 days after death 10–12 months previously. Following routine sectioning and staining with Harris's haematoxylin and eosin it was possible to determine the sex of these individuals from details of nuclear morphology, the distinctive mass of chromatin being particularly well seen in cartilage cells from female subjects.

To study the persistence of sex chromatin in unfixed tissues portions of foetal tissue were exposed to various environments, being placed (i) in water, (ii) in the open air, (iii) in a cellar, (iv) in an artificially heated atmosphere. Small pieces of skin were removed at regular intervals and fixed and stained as before. Sex chromatin was readily distinguishable until about the 15th day after death and in the case of tissue which had been immersed in water the sex could be determined until about the 23rd day. It was thought that cartilage cells might show persistence of sex chromatin for longer periods, but this was not established. Evidence for these findings were demonstrated and discussed.

APRIL 1956

A Special Meeting of the Society for the Session 1955–6 was held on Friday and Saturday, 27 and 28 May 1956 at Charing Cross Hospital Medical School, London, W.C. 2. The Chair at the various sessions was occupied by the President (Prof. R. D. LOCKHART) and the Vice-Presidents (Prof. R. E. M. BOWDEN and Prof. G. M. WYBURN), and the following are the authors' abstracts of the papers read.

The development of the prepuce in man. By T. W. GLENISTER.
Charing Cross Hospital Medical School

Thirty-eight human fetuses, ranging in size from 10 to 200 mm. crown-rump length, have been examined. It is concluded that the prepuce results from the combination of a preputial fold formation with the ingrowth of a cellular glando-preputial lamella. These processes are closely linked with the formation and fusion of the urethral folds on the glans. This fact accounts for the formation of the frenulum in normal males and the disposition of the prepuce in females and in cases of hypospadias.

The use of ^{35}S in cartilage homografts. By P. A. RING.
Charing Cross Hospital Medical School

The fate of homografts of epiphyseal cartilage of the rabbit has been investigated with the aid of radioactive sulphur. Litter mates aged 6 weeks were paired according to size. The distal end of the ulna was exposed in each rabbit and the epiphyseal cartilage, with a thin attached sliver of the adjacent epiphyseal and diaphyseal bone, was transferred from one animal to the other, care being taken to orientate the cartilage correctly in the host bone.

Radiographs of the forelimbs were taken after the operation and at the conclusion of the experiment. Twenty-five days after the operation each animal received an intraperitoneal injection of 5 mc. of ^{35}S . The animal was killed 24 hr. later and the forelimb fixed in formalin. Autoradiographs and ordinary sections of the epiphyseal cartilage were produced.

Although each of the homografts grew during the post-operative period there was considerable shortening compared with the control side. This shortening was associated with a degeneration of the cells of the reserve zone. ^{35}S was deposited only within the cartilage columns and was present in greater concentration than on the control side. The absence of ^{35}S from the reserve zone limits the value of this technique in assessing the fate of homografts of epiphyseal cartilage.

The effect of maternally administered cortisone upon the pancreas of the foetus.

By J. LEE and P. A. RING. *Charing Cross Hospital Medical School*

ACTH (12.5 mg., twice daily) and cortisone (4 mg./kg., daily) were given to rabbits during the last week of pregnancy. Specific changes occurred in the islet tissue of the foetuses. Most animals were killed on the 27th day, but a few proceeded to term. There was a high foetal loss, and few of the live-born animals survived.

Changes in the pancreas at the 27th day of gestation consisted of an increase in the size and maturity of the primitive islet tissue. In some foetuses the islets approached those of the adult in size and differentiation, but showed degenerative changes in the nuclei and cytoplasm of the cells. Occasional mitotic figures were seen. Changes after the administration of cortisone were more marked than after ACTH.

The pancreas of animals surviving into the postnatal period showed marked degenerative changes which appeared more obvious in the older animals. These changes appeared to progress to an almost complete islet atrophy.

It is suggested that these histological changes may be related to those seen in the pancreas of the foetus of the diabetic mother.

Fate and behaviour of the second set of subcutaneous corneal homografts in the guinea-pig. By P. BACSICH and G. M. WYBURN. *University of Glasgow*

The procedure in the experiments was as follows: a corneal homograft was inserted subcutaneously into the lateral body wall in fifteen host animals. Four weeks later a second corneal graft from the same donor was inserted into the same host animal, so that each of the fifteen animals had now two corneal homografts from the same donor. Both grafts were removed 3 weeks later. A description is given of the condition of both first and second sets of corneal grafts based on histological examination and on the assessment of the metabolism of the second set of corneal homografts as tested by the uptake of $^{35}\text{SO}_4$. From the data obtained from the above experiment there is no evidence of any sensitization of the host animal by a first set of corneal homografts.

Observations on the fixation of developing teeth.

By N. B. B. SYMONS. *University of St Andrews*

The presence of kionoblasts and radial cells in the ameloblast and odontoblast layers respectively, in the developing tooth of the rat, has already been demonstrated (Symons, *Brit. dent. J.* 98, 1955), employing 40% chilled formalin as fixative. This fixative has a pH of approximately 4.2. Further lowering of the pH produced by the addition of formic acid gives a less clear picture. The best result has been obtained with formalin neutralized by calcium carbonate (pH 6.4) not only for demonstrating the kionoblasts and radial cells, but also for general histological detail. A less good result is given by neutralization with magnesium carbonate (pH 7.6).

The kionoblasts and radial cells can be demonstrated in early developing rat teeth fixed in solutions containing osmium tetroxide, especially weak Flemming's fluid but with much less certainty.

Similar material has been fixed in Bouin, Zenker, Helly and absolute alcohol. Judged by the degree of shrinkage, only Helly's fluid produces a better picture of the developing tooth than absolute alcohol. It is thought that this shrinkage may be largely responsible for the inability to see the kionoblasts and radial cells, with such fixatives.

Using 40 % chilled formalin, these cells have also been found in early tooth germs in the cat.

Some observations on the remnants of human odontogenic and adjacent oral epithelium. By C. H. TONGE. *King's College, Newcastle upon Tyne*

The residuum of the odontogenic epithelium is generally regarded as a source of cells which may give rise to tumours of the jaws. An account is given of the arrangement of the remnants of the odontogenic and adjacent oral epithelium based on a study of serial sections of human embryos and fetuses ranging from 7 to 190 mm. c.r. length. At 7 mm. c.r. length the oral epithelium is of the low columnar type, whilst between 15–20 mm. c.r. length the characteristic tall columnar epithelium of the tooth-bearing area is seen. As progressive invagination of the odontogenic epithelium occurs the superficial part of the dental lamina is observed to be lined by epithelium similar in character to the adjacent oral epithelium. Between 60–100 mm. c.r. length, many ingrowths of the surface epithelium with localized mesenchymal condensations are seen, both labial and lingual in position to the dental lamina. The disintegration of the dental lamina, enamel organ and accessory ingrowths from the oral epithelium is traced. It is concluded that cysts, epithelial coils and cell islets are formed from the break up of the superficial part of the dental lamina and the accessory ingrowths from the oral epithelium. The deeper part of the dental lamina and the outer enamel epithelium, however, tend to fenestrate and form isolated clusters of epithelial cells which may disappear or remain latent with a proliferative potentiality.

The development of the human gubernaculum testis. By K. M. BACKHOUSE and H. BUTLER. *St Bartholomew's Hospital Medical College and University College of Khartoum*

Many accounts of the development of the gubernaculum testis are obscured by a confused nomenclature and a bewildering complexity of formal description.

In a 7.0 mm. c.r. length embryo, the mesonephros and adjacent body wall are covered by thick, columnar epithelial cells which gradually merge into typical flattened coelomic epithelium. With further development, the columnar epithelial cells become flattened, except for a band crossing the umbilical artery and extending from the gonad to the anterior abdominal wall. Beneath this cellular band is a residuum of dense mesenchyme extending from the gonad to the future scrotal region. This residuum is the primitive gubernaculum testis, whose intra-abdominal part, covered by columnar epithelial cells, becomes the plica gubernatrix. The plica is not yet developed as a ridge in a 16 mm. c.r. length embryo.

During the 16.0–23.0 mm. stage the plica gubernatrix begins to form a ridge as a result of the proliferation of the mesenchymal residuum. Ridge formation occurs along the whole length of the plica gubernatrix, but is most marked at the caudal pole of the mesonephros and at the junction of plica with anterior abdominal wall (the site of the, as yet undeveloped, internal inguinal ring). Felix (1912) interpreted these changes as indicating the formation of the plica gubernatrix by the fusion of two outgrowths—'plica inguinalis' and 'crista inguinalis'. Although it is convenient to divide the gubernaculum testis into three parts, intra-abdominal (=plica gubernatrix), intramural (=the part within the developing inguinal canal) and scrotal, these are parts only of a single continuous structure extending from the gonad to the scrotum. Associated structures (e.g. processus vaginalis, cremaster muscle, and inguinal canal) develop later in and around the primary mesenchymal residuum which constitutes the fundamental basis of the gubernaculum testis.

A histological study of bone growth using Alizarin Red A.S.By D. A. N. HOYTE. *University of Manchester*

Madder has been used extensively in studies of growing bones. Its usefulness is not, however, limited to macroscopic study, as clear evidence of its deposition can be seen in thin sections of undecalcified, unstained bone.

Alizarin Red A.S. was given by intraperitoneal injection to albino rats of various ages, the animals being sacrificed at intervals thereafter. Well-defined red lines can be seen and the details of ossification followed in the juxta-epiphyseal region, in the spongiosa of the metaphysis on endosteal and periosteal surface of the shaft, and in Haversian building sites. Areas of absorption and of stasis of bone are easily recognized. After this single injection, diffuse staining was not seen microscopically, either in bones from young or adult animals. When seen by the hand-lens or naked eye it is probably due either to the close aggregation of stained Haversian systems or to the red bone being seen through the thickness of white.

This technique gives results as good as those obtained by autoradiography.

The development of the hind-brain arteries in the rat.By D. B. MOFFAT. *University College, Cardiff*

Rat embryos of c.r. lengths between 4 and 10 mm. have been injected with indian ink via the heart or the dorsal aorta. After fixation, the vessels of the hind-brain region have been studied by dissection.

The most important artery supplying the hind-brain region at the 4-6 mm. stage is a lateral longitudinal vessel derived from the first dorsal intersegmental artery and running dorsal to the rootlets of the hypoglossal nerve. It is continuous caudally with a longitudinal anastomosis between the dorsal intersegmental arteries on the side of the neural tube. There are a number of connexions between the internal carotid arteries and the bilateral longitudinal neural arteries, and these are generally continued dorsally to join the lateral longitudinal vessel, the latter communications passing between the hypoglossal rootlets.

At the 6-8 mm. stage, the definitive posterior spinal plexus has formed, the previous anastomosis between the dorsal intersegmental arteries having disappeared. The cranial origin of the posterior spinal is the dorsal branch of the first dorsal intersegmental. The main supply to the lateral longitudinal channel is taken over by the most caudal of the anastomoses between the bilateral longitudinal neural arteries and the lateral longitudinal vessel, so that the latter will become a branch of the vertebral artery passing between the hypoglossal rootlets.

The significance of these and other findings are discussed.

The activity of the placodes during the development of the mixed cranial nerves in the sheep embryo. By E. H. BATTEN. *University of Bristol*

In fish and amphibian embryos the neural crest rudiment of the mixed cranial nerves is augmented by cells proliferated from certain placodal thickenings of the ectoderm which may furnish distinct lateralis and gustatory ganglia. While the existence of some placodes has been recorded in several mammals there is marked disagreement as to their participation in ganglion formation. It has, however, been insufficiently emphasized that the placodes in the mammal show intermittent proliferation so that cellular migration is transient, and indisputable evidence is not to be expected in every specimen. A study of placodal behaviour in forty-three sheep embryos has revealed reasonable evidence of a definite but limited contribution to the developing ganglia of the mixed cranial nerves.

The Gasserian ganglion develops largely from the neural crest, but receives a small contribution from minute thickenings and cellular spurs of the trigeminal placode. The epi-branchial placode of the facial nerve proliferates cells into the geniculate ganglion during

the 6–10 mm. stages. The epibranchial placode of the glossopharyngeal nerve sends a meagre contribution to the petrosal ganglion and then becomes associated with the developing carotid nerve. The large epibranchial placode of the vagus nerve becomes incorporated in the occluded cervical sinus attached to the nodose ganglion. During the 10–12 mm. stages it proliferates a mass of small cells which differentiate into neuroblasts by the 16 mm. stage. The fate of the migrant placodal cells received by the Gasserian, geniculate and petrosal ganglia remains uncertain, but it is suggested that they may differentiate into sheath cells.

The geometry and kinematics of the knee joint.

By C. C. D. SHUTE. *University of Cambridge*

The curvature and movements of the femoral condyles have been studied radiographically in a young adult male. Each condylar profile behind the patellar surface consists of two segments of logarithmic spirals, opening anteriorly and posteriorly, all of constant angle 75° . The spirals are set symmetrically on each side, but medially the anterior segment and laterally the posterior segment is the longer. During articulation with the tibia the change-over from one segment to the other on each side occurs at 30° flexion.

The paths traced out by the poles of the spirals indicate the relative amounts of rolling and gliding at various stages. Each condyle rolls in terminal flexion and the lateral condyle in terminal extension. The antero-posterior displacement of the condyles and the inter-condylar width are used to calculate the amount of longitudinal rotation. Rotation is greatest in terminal extension or initial flexion, but occurs throughout the movement except in final forced flexion. By 90° flexion sufficient rotation has occurred to offset the valgus angulation of the femur (12°). In full flexion the femur and tibia remain in alignment as a result of tilting of the condyles.

The excursion of the popliteus and ligamentous attachments is determined and the significance of the findings discussed.

Theoretical basis for segmental variations.

By D. B. ALLBROOK
and W. K. CHAGULA. *Makerere College, Uganda*

Homoeotic variations of vertebrae are known to be genetically determined in man and in various experimental animals (Kuhne 1932; Sawin, 1937; Green, 1950).

It is probable that similar segmental muscle variations are also genetically controlled (Little & Bragg, 1924). From experimental embryological work it is known that the presence of muscle fibres in some way 'attracts' growing peripheral nerve fibres (Detwiler, 1936). In the adult it is logical to presume that variation in the size of segmental nerves is indirectly dependent on the genetic mechanism which controls the segmental morphological development of sclerotome and myotome in the embryo.

This hypothesis is supported and illustrated by the findings in a cadaver (M.C. 296) in which there are present cervical ribs, eleven thoracic ribs, six lumbar vertebrae, cranial segmental displacement of various trunk muscles and prefixation of the brachial plexus.

The occurrence of a middle superior alveolar nerve in man.

By M. J. T. FITZGERALD. *University College, Cork*

From his studies by dissection and on dried bones, Wood Jones (*J. Anat., Lond.*, 73, 1939) concluded that a middle superior alveolar nerve was infrequent, and should not be considered a normal branch of the extracranial part of the maxillary nerve.

Observations on fifty dissected parts and on fifty dried bones indicated that the middle superior alveolar nerve appeared in some four out of five cases. Evidence of the 'classical' form was present in half the observed material; more than a quarter of the remainder indicated that a manifest variant of this type either was or had been present in the material

examined. Transillumination of the maxillary antrum *ab externo* was found particularly valuable in the investigation.

The morphological significance of these results was briefly discussed.

A comparison of human and anthropoid mesosterna.

By G. T. ASHLEY. *University of Manchester*

From a review of the literature and study of 1400 human and 239 anthropoid specimens, it is considered that, of the sterna under consideration, the chimpanzee sternum is the most highly specialized and least liable to variation; the sternum of orang-utan is least specialized and shows every indication of remaining primitive; and the sterna of gorilla, gibbon and man are in a plastic stage of phylogenetic development, and are therefore more subject to variation than those of either chimpanzee or orang.

New designations are introduced to indicate the main variations in form of human and anthropoid sterna.

The aberrant renal artery. By F. T. GRAVES (introduced by T. NICOL).

King's College Hospital, London

In 1953 the anatomy of the human intrarenal arteries was investigated in order to discover whether there was a constant pattern of these vessels. The arteries of post-mortem specimens were injected with a resin which subsequently solidified; the organic material was then removed by corrosion. The investigation (Graves, F. T., *Brit. J. Surg.* **42**, 132, 1954) showed that the distribution of the arteries within the kidney substance was constant and that upon this arterial basis the organ was divisible into five segments. It was also shown that there is no collateral circulation between the segments.

Although the initial work was done mainly as an aid to renal surgery, at the same time a study was made of the arteries of the renal pedicle, particularly those said to be 'aberrant arteries'. The investigation included the study of more than seventy plastic casts and more than sixty renal arteriograms. It was found that in those cases where the main stem of the renal artery divided into the intrarenal arteries at the hilum, i.e. the 'normal' arrangement, the artery to the lower segment most frequently took its origin from the main vessel at a point more proximal than any of the others. It was also found that when the renal pedicle consisted of more than one artery, the additional artery was one of the segmental arteries, frequently those to the apical and lower segments respectively, whose origin was more proximal than usual.

Further study of the so-called 'aberrant or accessory arteries' showed that these were in fact normal segmental vessels whose origin was more proximal than usual, being a variant of the normal, which might take their origin from the pedicle or from the aorta itself.

Like the normal segments of the kidney, the segments supplied by the 'aberrant' arteries had no collateral circulation between their areas, so that ligation of such a vessel would result in necrosis of the area supplied.

The embryology of the blood supply of the kidney as described by Felix has suggested that those intrarenal arteries which had a proximal origin at the hilum or from the pedicle were probably the result of a variation in the degeneration of the rete arteriosum urogenitale, whilst those which arose from the aorta were persistent mesonephric arteries.

The abnormalities in a human dicephalic tetrabrachiate monster.

By J. MCKENZIE. *University of Aberdeen*

Radio-opaque material was injected through the single umbilical vein and stereoscopic radiographs were taken. The thoracic and abdominal viscera were then removed through the anterior abdominal wall in order to preserve the skeleton.

Although the thoracic cavities communicated freely with one another, the heart and great vessels, the respiratory tract, thymus and oesophagus were complete on each side.

In the single large abdominal cavity there were separate, intestinal tracts as far down as the Meckel's diverticulum with the liver, pancreas and spleen duplicated. Rotation of the midgut had not occurred and the anus was imperforate; the right kidney was in the pelvis, but the corresponding suprarenal gland was normal in position. The arterial and venous abnormalities in the abdomen were also described.

Some observations concerning the function of the digital pacinian corpuscles (corpuscula lamellosa). By N. CAUNA and G. MANNAN. *King's College, Newcastle upon Tyne*

Pacinian corpuscles have been variously described as part of the lymphatic system, as glands, electric organs, peripheral ganglia, amplifiers of nerve impulses, organs of general sensation and pressure receptors. Suggestions have also been made that the corpuscles may play some part in the regulation of the peripheral circulation, since they are found in association with blood vessels.

The present work is concerned with the arrangement of vessels in human digital Pacinian corpuscles.

It has been found that groups of corpuscles are usually closely associated with the glomerular arterio-venous anastomoses. The outer bulb of the corpuscle contains long interlamellar vessels of up to 50μ in diameter. They are supplied by small arteries and drained by veins which join the outlet of the adjacent arterio-venous anastomosis. It is apparent that the venous drainage of the corpuscle is dependent upon the state of activity of the anastomosis. When the anastomosis opens and the arterial blood fills its outlet, the flow of blood from the Pacinian corpuscle may be obstructed or even reversed. As a result the pressure inside the corpuscle may be raised and transmitted to the receptors of the inner bulb.

It is suggested that the digital Pacinian corpuscles may provide a mechanism for recording changes in local blood pressure due to the action of the arterio-venous anastomosis. The significance of the vessels and the numerous layers of the corpuscle can be explained by this suggestion.

Receptor nerve endings in the adult dog heart.

By R. L. HOLMES. *University of Leeds*

Various types of subendothelial afferent nerve endings of the heart and great vessels have been described. Vital-staining methods often demonstrate a nervous network, while after silver impregnation branching nerve endings have been reported.

Receptor areas in the walls of the atria and great veins of the adult dog heart have been localized physiologically (Coleridge, Hemingway, Holmes & Linden, *Proc. Physiol. Soc.*, April 1956). These regions from the hearts of the experimental animals were later sectioned serially and examined after silver impregnation. Nerve fibres were seen in all layers of the walls. In the subendothelial layer scattered circumscribed nervous structures were observed, consisting of thick fibres, branching into finer filaments, apparently ending in close relation to associated cellular elements. A single thick nerve fibre could often be traced to each terminal area. Although sometimes lying close to smooth muscle cells in the endocardial tissue, these endings did not appear to innervate them; neither were they connected with other nervous structures except by the deeper-lying fibres.

Single nerve filaments or small bundles of fibres were also seen, lying especially in the deeper layers of the connective tissue, and along its junction with the main muscle coat.

It was considered that the nervous structures described, occurring in physiologically demonstrated receptor areas, might be specialized afferent nerve endings.

The fine structure of the chorioidal epithelium in the rabbit.

By J. W. MILLEN and G. E. ROGERS. *University of Cambridge*

Observations of the finer details of the structure of the chorioidal epithelium by routine histological methods is limited by the resolving power of the microscope. An investigation of the intracellular morphology has therefore been carried out with the aid of the electron-microscope in order to determine to what extent it resembles or differs from that of other cells whose function is more fully understood.

Portions of chorioid plexuses from the lateral ventricles of young rabbits were fixed immediately after death in 1 % buffered osmium tetroxide. After dehydration with alcohol, sections were cut and mounted on carbon films for examination with the electron-microscope.

The internal structure of the chorioidal epithelial cells is in general similar to that observed in cells from other tissues: mitochondria with typical internal structure, double membranes and intracytoplasmic vesicles have all been seen. The cells show infolding of the basal plasma membrane comparable to that found by other workers in the epithelium of the renal convoluted tubules, acinar cells of the pancreas, and in the ciliary epithelium of the eye. The chorioidal epithelial cells bear cilia whose internal structure is similar to that described for cilia elsewhere. Curious vesicular structures whose nature is unknown are also found in the basal parts of the cells.

Whilst the results of this work do not enable an unequivocal answer to be given concerning the mechanism of the production of the cerebrospinal fluid it is considered that they provide a basis for further investigations of the chorioid plexus under differing physiological conditions.

The glycogen body of the chick spinal cord. By A. D. DICKSON

and J. W. MILLEN. *University of Cambridge*

The glycogen body, which is peculiar to birds, is an ovoid mass of cells situated on the dorsal aspect of the lumbo-sacral spinal-cord. Its function is not known.

The meningeal relationships of the glycogen body have been investigated by many workers, but still remain in doubt. There are two more or less distinct schools of thought. One holds that the body is enclosed between two layers of meninges which correspond to the pia and arachnoid membranes. The other school holds that it is entirely submeningeal in position. Whilst the origin of its cells is variously stated to be in the meninges or in the wall of the spinal cord, both schools agree that the central canal passes through the ventral part of the glycogen body.

An investigation has been carried out in chicks, using the Millen and Woollam modification of Long's reticulum stain, in an attempt to throw more light on the meningeal relationships of the glycogen body and on the site of origin of its cells. The results of this work suggest that, in the definitive condition, the glycogen body consists of a larger dorsal portion and a smaller ventral portion, which are not distinguishable from one another by the staining reactions of their cells. The smaller portion surrounds the central canal of the spinal cord and is continuous with the neural tissue. The larger dorsal portion lies between two lepto-meningeal layers which fuse at its periphery. This work further suggests that the cells of the glycogen body differentiate in the wall of the spinal cord.

The fine structure of adreno-cortical mitochondria.

By J. D. LEVER. *University of Cambridge*

The large number of mitochondria normally present in the adrenal cortex is reduced after hypophysectomy and increased on ACTH stimulation. From an electron microscopic study, the internal membranes of adreno-cortical mitochondria, are arranged either as filaments or sacs in the *z. fasciculata* and *reticularis*: but in the *z. glomerulosa* these membranes, though usually filamentous, may appear as cristae. On activation of this zone

by depression of the Na/K ratio the mitochondrial membranes became exclusively saccular. Bodies equivocal in appearance between mitochondria and lipid droplets are depicted. Broken disorganized, mitochondria, seen largely in juxta-medullary cells, suggest that these bodies are readily expendable. Evidence suggesting a mode of genesis for adrenocortical mitochondria is presented.

The development of the spinal cord of *Xenopus laevis*.

By ARTHUR HUGHES. *University of Cambridge*

The literature on the neuro-embryology of Amphibia is far more extensive for the Urodeles than for the Anura. Of the latter, *Xenopus* has not hitherto been studied, although the morphology of the larva has been described by Weisz in 1945. This animal presents several remarkable features in development, principally related to the ciliary feeding mechanism of the larva. In the development of the spinal cord, the Rohon-Beard cells, the giant neurones which form the first sensory system, are at first dorso-lateral in position, but soon move medially to the mid-line. Other neurones derived from the neural crest remain outside the cord in the median plane. The sensory system of the early larva of *Xenopus* thus resembles that of fishes such as *Lophius*, and may be unique among tetrapods.

These and other features of the developing cord are being studied by several methods, including ultra-violet microscopy, mainly with regard to the changes which occur within the developing neurone. *Xenopus* is particularly suitable for such studies in that for the greater part of larval life the grey matter of the cord consists only of two layers of cells, one ependymal and an outer one of differentiated neurones, motor and internuncial, together with the Rohon-Beard cells.

Anatomical localization of excitation and inhibition in the spinal cord.

By J. M. SPRAGUE (introduced by J. D. BOYD). *University of Cambridge*

Degeneration of dorsal root fibres into the lumbosacral spinal cord was followed 3-5 days after extradural root section in the cat. Frozen sections have been stained with the Laidlaw modification of the Nauta technique, which selectively reveals degenerating axoplasm up to, and sometimes including, the synaptic terminals. Usually, however, 'boutons' themselves cannot be identified on the beaded or disintegrating non-medullated segment. Particular attention was paid to the distribution of those root fibres which make direct contact with somatic motor cells. Most of this monosynaptic degeneration following section of a single root was limited to the ventral horn of the same segment, but it occurred in lesser amounts throughout the lumbosacral enlargement (L4-S2). In areas two segments distant from the cut root, degeneration was largely limited to the dendrites of the motor nuclei. In contrast, degeneration close to the cut was found on both cell bodies and dendrites of motoneurons. This anatomical finding was correlated with physiological studies on the segmental distribution of facilitatory and inhibitory effects on test monosynaptic reflexes in the lumbosacral cord. Thus facilitation and excitation was associated with axosomatic and axodendritic terminals, while inhibition was spatially related to axodendritic terminals alone. Most of the dendrites on which the inhibitory fibres ended could be satisfactorily identified as belonging to motoneurons, but some contribution from neighbouring interneurons could not be ruled out. This evidence supports the contention that at least part of short latency inhibition is monosynaptic, and suggests a cytological localization of inhibition and excitation in this pathway.

A quantitative study of the fornix-mamillothalamic system of connexions.

By W. M. COWAN, T. P. S. POWELL and R. W. GUILLERY. *University of Oxford and University College, London*

Counts have been made of the fibres in the post-commissural fornix (at the level of the anterior commissure and immediately rostral to the mamillary bodies), of the cells in the medial mamillary nucleus, of the fibres in the mamillothalamic tract, and of the cells in the three anterior thalamic nuclei in the rat, rabbit, monkey and man.

The post-commissural fornix loses about 50 % of its fibres between the commissure and mamillary nuclei. The number of fibres in the premamillary fornix, medial mamillary nucleus and mamillothalamic tract is of the same order in the rabbit, cat and monkey (80,000–120,000). The total number of cells in the three anterior thalamic nuclei in these three species is again of the same order (120,000–140,000). The figures for the rat are much lower (premamillary fornix, 20,000; medial mamillary nucleus, 35,000; anterior nuclei, 65,000) and for man considerably higher (390,000 mamillary cells; 680,000 anterior thalamic cells).

Thus, whereas the ratio between the number of cells in the medial mamillary nucleus and the number in the anterior thalamic nuclei is of the order of 1:1.5 or 1:2 in all species, the ratio between the number of fibres in the premamillary fornix and the cells in the mamillary nucleus varies from 1:2 (rat); 1:1 (rabbit, cat, monkey) and 2:1 (man).

The number of cells per unit volume in the medial mamillary and anterior thalamic nuclei has been found to vary inversely with the total brain weight.

A histological study of some commissural and septal connexions of the hippocampus in the rabbit. By B. G. CRAGG and L. H. HAMLYN. *University College, London*

Lesions were made in the fimbria for investigation of the ventral commissure and in the angular tract of Cajal near the midline for the dorsal commissure. In the controls the appropriate structure was exposed but not cut. The extent of the lesion was in all cases checked histologically. After survival times of 5–7 days the course of the fibre degeneration was studied using the Nauta technique. Following section of the fimbria on one side degeneration was found in the posterior half of the fimbria on the opposite side. This degeneration extended into fields CA3 and CA4 of the Ammon's horn. In the ipsilateral hemisphere lateral to the lesion, as well as the commissural degeneration, there were degenerating fibres present throughout the anterior as well as the posterior half of the fimbria and these extended back over the dorsal alveus to the presubiculum where they were present in all its layers. These fibres could have come from the septal nuclei. After lesions of the angular tract, degenerating fibres could be traced from this tract to the upper entorhinal cortex in both hemispheres. The degeneration was sharply limited to this cortical area and extended throughout all its layers.

An electrical study of some of the commissural and septal connexions of the hippocampus in the rabbit. By B. G. CRAGG and L. H. HAMLYN. *University College, London*

The criterion used to decide electrically that a tract is afferent to a nucleus is that stimulation of the tract should produce a large and early response in the nucleus that varies in depth in a manner compatible with the membrane hypothesis and the histological structure of the nucleus. It has been found that later responses occur in secondary orders of neurones, and are greatly enhanced by repetition of the stimulus. The findings are as follows:

The dorsal commissure receives fibres from the presubiculum and crosses the midline to terminate in part of the entorhinal area. Stimulation of the commissure evokes a primary response in the entorhinal area and a secondary response in the dentate fascia. Stimulation of the presubiculum evokes a primary response in the ipsilateral dentate fascia and a secondary response in the Ammon's horn.

The ventral commissure occupies the posterior part of the fimbria and produces a primary response in the region of the dentate fascia and area CA₄. Stimulation of the commissure can evoke secondary responses in all the fields of the Ammon's horn. Stimulation of the anterior part of the fimbria evokes primary responses in the septal nuclei. Stimulation of the latter evokes a primary response in the presubiculum. With the exception of the entorhinal area, all the structures concerned have been exposed for stimulation and recording.

Differences in the regenerative power of afferent and efferent fibres in the optic nerve of anurans. By H. MATURANA (introduced by J. Z. YOUNG). *University College, London*

In amphibians section of the optic nerve is usually followed by successful regeneration and recovery of vision. Two sorts of fibres are involved: afferent fibres from the retina to the tectum and efferent fibres to the eye, which probably come from the tectum itself, following the optic tracts. These two groups of fibres do not have the same regenerative power, as is shown by the difference in appearance and degree of success in their regenerative attempts. The afferent axons from the retina ramify profusely as many penetrating fine fibres which quickly reach the central stump, or grow into the surrounding tissues when a piece of nerve has been resected. Small and transitory dilatations may be produced in the presence of obstacles. The efferent fibres, on the other hand, form end-clubs from the very beginning, with little or no branching, whether or not there is a gap between the two stumps. These fibres seem to have little or no regenerative power. The club-ends are found with no further modification 50 days after lesion and after 110 days the clubs are still present, although they may be less in number. It seems probable that the majority, if not all, of these processes of central neurons fails to produce effective regeneration. Visual recovery begins about 50 days after section of the optic nerve and progresses with variable speed to be essentially complete at about 110 days, as far as can be judged by the response to a moving lure. Nevertheless, this recovery is not absolute and several degrees of deficiency can be observed. Whether the failure of regeneration of the efferent fibres affects the visual recovery is not yet fully clear, but this situation offers an opportunity to study their influence on retinal activity and visual behaviour in general.

Observations on the distribution of extrinsic nerve fibres in the gut of the rat. By GRAEME SCHOFIELD. *University of Oxford*

In normal rats, in addition to the fine fibres which terminate in the enteric ganglia, relatively thick fibres pass through the ganglia without the intervention of a synapse to enter the mucosa. Terminal swellings and beaded fibres occur only infrequently in the myenteric plexus.

In experimental animals a numerical increase in terminal swellings, when compared with normal rats, associated with marked beading and fragmentation is taken as evidence of degeneration in sectioned nerve fibres. The following changes have been observed in the enteric plexus at intervals up to 10 days after operation.

(1) After unilateral high cervical vagotomy degenerative changes occur in the myenteric but not in the submucous plexus. These changes are maximal 48–72 hr. after operation and extend from the oesophagus to the mid-colon.

(2) After unilateral vagotomy associated with extrinsic denervation of a loop of jejunum, the latter shows degenerative changes in both the myenteric and submucous plexus within 24 hr.

(3) After division of thoracic nerve roots at the spinal cord, degenerative changes occur in the myenteric plexus.

(4) Section of nerves passing to a loop of jejunum results in partial denervation only. Five days later relatively thick fibres occur in the mucosa from which fine invading fibres extend towards the epithelium.

The significance of these findings is discussed.

The histochemical demonstration of tyrosinase in the melanocytes of normal, caucasian epidermis and the numerical relationship of tyrosine-positive and dopa-positive cells. By GEORGE SZABÓ. *London Hospital Medical College*

The presence of tyrosinase in the melanocytes of normal, Caucasian epidermis has not been shown before histochemically. Its presence was inferred from biochemical investigations and from the fact that melanocytes utilize dopa. It was thought that the enzyme was inhibited, melanocytes being active only when irradiated or in neoplastic state.

By using pure epidermal sheets obtained by the skin splitting technique of Medawar, we found, however, that in many regions of the body skin the melanocytes utilized tyrosine to form pigment granules. The perifollicular and the 'giant' melanocytes were most active. The cell counts of tyrosine-positive and dopa-positive melanocytes were the same in several regions of the body—these included cheek, forehead, ear, nasal mucosa and scrotum. In other regions (penis, chest, neck and arm), however, the number of tyrosine cells was less than those of dopa-positive melanocytes. Some specimens from the thigh did not show any sign of positive reaction.

It was, therefore, demonstrated histochemically for the first time that pigment formation occurred in normal melanocytes by the utilization of tyrosine as substrate. Furthermore, the same cells which were dopa positive might also be tyrosine positive. The regional difference in tyrosinase activity might be the result of several factors. In the exposed regions the solar irradiation was an activator. In the genital regions the melanocytes might be of a different genetical type from those of the general body surface. There might be a regional variation in the suggested inhibitory action of sulphhydryl groups. If Foster's hypothesis of high tyrosinase activity in an area densely populated by melanocytes should be true (*Genetics*, 38, 662, 1953), these density factors might be important also. Certainly, the most strongly positive areas, including the perifollicular regions, were densely populated.

JUNE 1956

The Summer Meeting of the Society for the session 1955-6 was held on Friday and Saturday, 29 and 30 June 1956 in the Department of Anatomy, The Queen's University, Belfast. The President (Prof. R. D. LOCKHART) was in the Chair, except for one short period during the morning session on Saturday, 30 June, when Prof. G. M. WYBURN presided.

The following are the authors' abstracts of the papers presented.

Induction of cartilage and bone by devitalized implants under the kidney capsule of rabbits. By J. B. BRIDGES and J. J. PRITCHARD. *Queen's University, Belfast*

In a previous communication we reported the regular occurrence of bone formation in and around implants of alcohol-killed callus and epiphyseal growth cartilage placed under the kidney capsule of rabbits. We now find that implants of living urinary bladder likewise induce bone in this situation. On the other hand, alcohol-killed urinary bladder and a number of other tissues so devitalized primarily induce cartilage when implanted under the kidney capsule, with bone formation as a later and secondary phenomenon. Of all the tissues implanted, striated muscle, especially that from the tongue, showed the greatest cartilage-inducing activity. Surprisingly, alcohol-killed skeletal tissues other than hypertrophic cartilage failed to induce either bone or cartilage. Alcohol alone, and alkaline phosphatase pellets, likewise, had no inducing effect. Whatever may be the nature of these cartilage and bone inductors, it seems certain that there is no relationship between the alkaline phosphatase content of a tissue and its capacity to induce bone or cartilage under the conditions of this experiment.

Growth of the cranial base. By J. H. SCOTT. *Queen's University, Belfast*

In order to analyse the growth of the cranial base in man the usual division into two segments, nasion to pituitary fossa, and pituitary fossa to basion, is inadequate. If the anterior segment is subdivided into a frontal region from nasion to foramen caecum and a sphenoid region from foramen caecum to the pituitary fossa, it will be seen that the former continues to increase by surface deposition at nasion until adult life is reached, while the latter attains adult dimensions about the seventh year. The cribriform plate region is the most stable area in the cranial base after the seventh year, and the midline floor at the anterior cranial fossa is the most suitable region for the superimposition of cephalograms in serial studies of cranio-facial growth (De Coster, 1952). The point nasion migrates forwards and upwards during growth, and this migration makes the line nasion to pituitary fossa an unsuitable base line for growth studies. Furthermore, this process of migration must be kept in estimations of changes which take place in the angulation of the cranial base.

Duplication of stomatodaeal structures in a male infant.By W. R. M. MORTON. *Queen's University, Belfast*

A full-term male child, who lived for 3 days, exhibited duplications in the oro-pharyngeal region of the brain which must have arisen prior to the breakdown of the stomatodaeal (oral) membrane. The main anomalies consisted of a double frenulum in the upper jaw with two intervening accessory central incisor teeth; complete cleft palate behind the alveolar arch; two lower lips; partial duplication of each half of the mandible and tongue; two separate pituitary glands suspended side by side from the brain by separate stalks, and lying behind a rather wide optic chiasma; a bar of tissue, running from the angle between the bifid front part of the tongue in the floor of the mouth, upwards to join the roof of the nasopharynx; and a hair-covered polypoid mass attached to the septal region of the nose just anterior to this bar. The nature of the duplications and the position of this bar of tissue suggest that the anomalies arose as a result of an abnormal invasion by mesoderm in the midline of the stomatodaeal membrane before the pituitary had differentiated. No references to comparable duplications of these structures have been found in the literature.

Sacral growth in the rat. By T. J. HARRISON. *Queen's University, Belfast*

The mode of sacral growth in the rat has been investigated (a) by taking measurements from a series of radiographs of individual animals during the growth period (24 animals), (b) by measurements of autoradiographs made from 17 rats fed with ^{45}Ca , (c) by measurements of growth after the insertion of metallic markers in the sacrum (4 animals), and (d) by histological examination of paraffin sections (10 animals).

It was found that (1) the sacrum forms a constant proportion (18 %) of the length of the vertebral column (excluding the tail) throughout the period from 17 to 248 days; (2) sacral length increases at a constantly greater rate than sacral breadth; (3) growth in length of the sacral bodies is twice as rapid at the caudal end as compared with the cranial end; (4) growth in sacral breadth occurs mainly just beneath the sacro-iliac joint; and (5) the centrum and neural arches synostose during the 3rd and 4th weeks after birth, at which time secondary centres appear in the intervertebral discs between the sacral bodies.

The fine structure of lamellibranch muscle.By J. BOWDEN. *Queen's University, Belfast*

Lamellibranch muscles fall into three main groups, viz. (1) smooth, (2) transversely striated, (3) so-called 'double obliquely striated'. The double oblique striations are here shown to be artefacts produced by the severing of the muscle from its shell attachments, and are similar to the 'contraction bands' of smooth muscle. It can be shown by the com-

parison of stained sections with theoretical models that these 'contraction bands' are arranged in helicoids, but the reason for this is unknown. The transversely striated fibres are essentially similar to those of arthropods and vertebrates, and show progressive changes during contraction similar to those described by Hansom & Huxley (*Nature, Lond.*, **173**, 1954). Unexpectedly, most apparently transversely striated fibres on closer examination show unmistakable evidence of a helicoidal organization in the striations like that described by Tiegs (*Phil. Trans. B*, **238**, 1955) in insect muscle.

Bone induction in experimental renal ischaemia.

By J. B. BRIDGES. *Queen's University, Belfast*

It has long been known that bone may form in a rabbit kidney after ligation of the renal artery. This has been confirmed and shown to occur also in the rat and guinea-pig. Bone begins to form 2-3 months after operation in the dense connective tissue immediately adjacent to the surviving epithelium of the renal pelvis and spreads towards the cortex, replacing calcified necrotic tubules. Normal bone marrow makes its appearance later. No cartilage is seen at any stage. Bone formation does not occur in the testis or spleen after tying the respective arteries. It is concluded that the transitional epithelium of the renal pelvis contains a specific bone inductor in these animals.

A film illustrating some stages of parturition in the fruit-bat, *Pteropus giganteus*. By H. HUGHES. *University College, Cardiff*

This film shows mating behaviour and the events of parturition in the bat. Particular attention is drawn to the mature stage in which the young is born, the post-natal care of the mother to the young, and the stages of activity which the newborn exhibits in clinging to the mother, especially to the nipple.

Cells in the spiral arteries of the pregnant uterus. By J. D. BOYD, *University of Cambridge*, and W. J. HAMILTON, *Charing Cross Hospital Medical School, London*

The spiral arteries that open into the intervillous space during pregnancy undergo remarkable structural alterations. Accompanying these changes cells appear in the lumina of many of the vessels. These cells are certainly, in part, of cytotrophoblastic origin, but some of them may be of decidual origin. Often the lumen of a spiral artery may be completely blocked by such cells. In many vessels, however, the cells are arranged quite loosely and the question arises as to why such cells are not dislodged by the flow of blood in the arteries. The fact that cytotrophoblastic cells migrate up the arteries, against the flow of the blood, but that they have never been observed to enter the uterine venous openings from the intervillous space is also noteworthy.

The pharyngeal hypophysis. By J. D. BOYD. *University of Cambridge*

Observations are reported on the structure and nerve supply of the human pharyngeal hypophysis. The structure is derived from the pharyngeal extremity of Rathke's pouch. It grows during foetal life, usually possesses a rich vascularization and closely resembles the adenohypophysis. In foetal material it has been found to possess a nerve supply derived from the sphenopalatine ganglion. The pharyngeal hypophysis appears to be constantly present in man and its cells can differentiate as do those of the adenohypophysis. There is no positive evidence that it possesses a physiological function.

The vascular pattern of the post-partum rat uterus.By A. YOUNG. *University of Glasgow*

The changes in the vascular pattern of the Albino Wistar Rat during the post-partum period have been followed in a series of twenty-five animals by means of injections of radio-opaque media.

Radiographically, the vascular pattern of the implantation sites has returned to normal by 8 days in a lactating animal. Non-lactating animals appear to require a somewhat longer time.

Splenic venography in the rat. By J. L. BRAITHWAITEand D. J. ADAMS. *University of Liverpool*

The exit of radiopaque media from the spleen has been studied extensively in the rat, following their injection directly into the spleen or retrogradely along the splenic vein or one of its tributaries.

Initially all the vascular pedicles of the spleen (of which there are normally five or six) have been left intact; the channels of drainage and the time taken for the medium to disappear have been studied particularly. This has been followed by interruption of one or more vascular pedicles and subsequent injection into the avascular zone.

The venous compartments of the spleen, previously described, are confirmed in that an injection into an intact zone is ultimately drained by its parent vein without diffusion into the vessels of adjacent compartments. Obstruction to this portal of exit is followed by drainage along the relatively large anastomotic channel (which links together the smaller venous radicles in the individual compartments) and not by diffusion through the pulp spaces of one segment and those of the next.

The mechanism of drainage is discussed.

Intravital staining of the rat testis with trypan blue.By J. S. BAXTER. *University College, Cardiff*

Trypan blue is stored in particulate form by cells in the intertubular tissue of the adult rat testis. Cappell (1929) has claimed that these are interstitial cells, thus following earlier workers on this problem. The juvenile rat testis shows similar dye-staining cells in the intertubular tissues. It seemed on general grounds very doubtful that cells of the reticulo-endothelial system could be more than phagocytic cells, and so interstitial testicular cells in the juvenile and adult animal would have different morphological and functional characters. These latter cells have been studied to find out whether they belong to the R-E system or not.

By various methods of staining for fat and ketosteroids it is evident that the dye-staining cells are not interstitial cells in spite of the fact that they are very evident in the testis just after birth.

The fate and significance of the primary bony cuff of the mammalian long bone. By C. W. M. PRATT. *University of Cambridge*

A method is described whereby in the usual histological preparations of mammalian long bones it is possible to interpret the pattern of growth and recognize the onset of erosive processes. The method is based upon the fact that the primary bony cuff formed by the perichondrium continues to elongate throughout the growing period of the bone. The fate of the cuff is described in the femur of the rat, where the appearance of a secondary marrow cavity results in the absorption of the central portion of the cuff. As the marrow cavity enlarges, the cuff becomes restricted to the metaphysal region where it separates the

endochondral bone from the periosteal bone. Remodeling of the metaphysis may result in further reduction of the cuff due to absorption of its diaphysial border. The changes in the width of the bone at the level of the epiphysial plate, that have been occurring during growth, are clearly indicated by the appearance of the cuff.

Adaptations in the skin of the common seal (*Phoca vitulina*). By W. MONTAGNA and R. J. HARRISON. *London Hospital Medical College*

The skin from a number of newborn, adolescent and adult common seals has been studied to see in what ways, if any, its morphological and histochemical characteristics differ from those of other furred mammals, since it might be expected that the skin of the seal would possess certain adaptations for protection against the injurious action of water. The details of some of these adaptations seem to be self-explanatory, but those of others give so far no clue to their significance.

The surface epidermis is very thick and its stratum corneum is compact and imperfectly keratinized, in contrast with that of land mammals, which is well keratinized and flaky. The hairs of the seal grow in groups of five or six; one of these is a large guard hair, the others are small underhairs which fill the space at the base of the large one. The follicles of each group are conjoined and the hairs share a common pilary canal. The guard hairs are flattened horizontally and lie over each other like imbricated scales. As in all other mammals studied, the outer sheath of growing hair follicles is laden with glycogen, that of resting follicles is not; the dermal papilla of growing follicles stains metachromatically and is Schiff-reactive; the papilla of resting follicles lacks these properties. The numerous large sebaceous glands seem to be very active, and are rich in esterases and in alkaline phosphatase activity. Each hair group possesses one sweat gland, which enters the pilary canal together with the sebaceous glands. The sweat glands have the characteristic features of apocrine glands in other mammals, and they are weakly reactive for succinic dehydrogenase activity, but strongly reactive for esterases and alkaline phosphatases. The glands seem to secrete small amounts of a viscid material which is not readily miscible with the sebum, and which seems to be of a mucoid nature.

The dermis possesses two curious features: (1) the upper part of the reticular layer and the papillary layer are riddled by sinusoidal venules and capillaries, and (2) there are no mast cells present.

Observations on the mucus-secreting cells of the alimentary tract and associated glands. By B. F. MARTIN. *University of Sheffield*

A comparative study was made of the mucus-secreting cells of the alimentary tract and associated glands of the rabbit, guinea-pig, rat, mouse, cat and dog. Both the periodic acid-Schiff (PAS) method and the classical mucicarmine staining procedure were employed, and a comparison made between the results obtained with the two techniques. The results were not identical for all tissues studied, even when glycogen (demonstrable with PAS but not with mucicarmine) was removed from the sections by the action of saliva.

Observations of particular interest were made on the salivary glands, which vary in type from species to species, and on the pattern of goblet cell distribution in the large intestine.

The post-natal fate of the abdominal extra-adrenal chromaffin tissue in rodents. By REX E. COUPLAND. *University of Leeds*

The distribution of chromaffin tissue in the rabbit, mouse, guinea-pig and rat has been studied. In spite of many accounts to the contrary, phaeochromocytes are associated with the pre-aortic sympathetic plexus of the rat, but are few in number.

In the rabbit, mouse, and guinea-pig extra-adrenal chromaffin tissue is abundant at about the time of birth and increases in amount during the first few weeks of post-natal

life. In young animals large discrete para-aortic bodies lie in the pre-aortic region and small groups of chromaffin cells are also found in the lumbar para-vertebral ganglia.

In these rodents the supporting connective tissue stroma of the para-aortic bodies increases in amount with age and there is evidence of distraction.

In the newborn mouse a large discrete para-aortic body lies near the left renal vein, this completely disintegrates as the animal grows and no longer forms a discrete unit after the age of two months. The chromaffin cells become scattered throughout the lower pre-aortic region, but there is no evidence of a peripheral migration of chromaffin cells.

Cellular infiltration is never associated with the above age changes.

Advances in microradiography and X-ray microscopy.

By G. A. G. MITCHELL. *University of Manchester*

X-rays have greater penetrating power and have shorter wavelengths than those of visible and ultra-violet light, and theoretically they should have more resolving power as the revelation of fine detail in any system producing magnification depends ultimately on the wavelengths of the rays used. There are great technical difficulties involved, however, in exploiting the full potentialities of X-rays in microscopy, although some promising methods, of overcoming these are now being developed by physicists. These are described briefly, before showing results obtained by refinements in contact microradiography, the only method readily available so far for biological workers.

Investigation of tissue fluorescence induced by acridine orange.

By J. A. ARMSTRONG. *National Institute for Medical Research, London*

Fluorochrome dyes are much recommended in the German literature as an alternative to orthodox staining methods for plant and animal tissues. The basic substance acridine orange, when used as a vital stain, is said to indicate the viability of cells. However, these applications of fluorescence microscopy have not been widely adopted, possibly because their use is largely empirical and the significance of differential colour fluorescence in tissues has not been established.

This communication concerns initial results in a study of the fluorescent properties induced by acridine orange in fixed animal tissues. Attention has been given to the possible influence of such factors as fixation, pH control and dye concentration in staining, and wavelength of the light used for activation. Under controlled conditions clear and repeatable results are obtained, different cells and cell components showing characteristic fluorescent colours. Moreover, there is evidence that with appropriate pH control one can identify and distinguish between intracellular localizations of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The effect of radon exposure on the developing maxilla.

By J. MCKENZIE, J. F. PHILIP and D. LOGIE. *University of Aberdeen*

A child of 3 months was treated by radon needles for a highly malignant tumour on the dorsum of the tongue. At the age of 12 years she now shows gross maldevelopment of the hard palate and alveolar margin with accompanying changes in the rest of the maxilla and mandible. Many teeth have failed to appear and those which have erupted are poorly formed.

The different effects of the radon therapy on the cartilaginous and periosteal growth in the maxilla are described and the associated changes in the other bones are explained.

Leprosy as an aid in the investigation of the neurohistology of skin.

By G. WEDDELL, ELIZABETH PALMER and G. C. SCHOFIELD. *University of Oxford*

For purposes of diagnosis and to assess the effect of treatment, it is customary to examine sections taken from the margin of affected zones of skin in leprosy. Thus there is available to us a source of both normal and pathological fresh skin from different regions of the body in numerous subjects of all ages. Sections from this material have been: (1) impregnated with silver; (2) stained with Masson's trichrome stain; (3) stained by methods designed to demonstrate mycobacteria leprae selectively; (4) impregnated with silver and subsequently stained selectively for mycobacteria leprae.

Preliminary investigations have shown: (1) that the course and distribution of nerves in normal skin varies greatly from region to region of the body, attempts are being made to express these differences in relation to the density of innervation; (2) that there is a specific relationship between the mycobacteria and the cutaneous sensory nerve fibres; (3) that, at certain phases in the disease process, the mycobacteria are so closely confined to the neighbourhood of nerve fibres that the patterned arrangement of the nerves in the skin can be inferred in the absence of a specific nerve stain.

The tectal projection to the brain stem reticular formation in the cat.

By G. W. PEARCE (*Queen's College, Dundee*) and P. GLEES (*University of Oxford*)

In a previous communication we emphasized the importance of the tecto-reticular projection and, using Marchi stained material, showed that over one-half of the fibres of the crossed tecto-spinal tract end in the brain stem and chiefly in the reticular formation. The present paper is an account of the connexions of the superior corpus quadrigeminum with the reticular formation nuclei as outlined by Meessen, Olszewski & Baxter (1949, 1954).

The brain stems of over twenty cats, after survival periods of 4–28 days following lesion of the superior corpus quadrigeminum, have been examined by the methods of Marchi, Glees and Nauta.

In the midbrain on the same side as the lesion, the nuclei cuneiformis and subcuneiformis receive a large projection from collaterals of tecto-spinal fibres and from direct tecto-reticular fibres. A few fibres reach the nucleus cuneiformis of the other side via the collicular commissure.

Distal to the tecto-spinal decussation the crossed tecto-spinal tract gives fibres chiefly to the nucleus pontis centralis and caudalis and to the nucleus gigantocellularis on the side opposite to the lesion. The ventromedial parts of these nuclei are especially involved and there is some evidence that a few fibres may cross the midline to end in the corresponding nuclei on the same side as the lesion.

The topographical distribution of reticular nuclei receiving fibres from the tectum is very similar to that of areas receiving fibres from the cortex (Rossi & Brodal, 1956) and to those said to influence cerebral activity.

The treatment of hydrocephalus, in rabbits, due to maternal hypovitaminosis A.

By J. W. MILLEN and A. D. DICKSON. *University of Cambridge*

The occurrence of hydrocephalus in the young of female rabbits suffering from a gross vitamin A deficiency has been described in previous communications. It has also been reported that one of the early signs of hypovitaminosis in the young is an increased cerebro-spinal fluid pressure.

The present communication is concerned with the effects produced by the administration of vitamin A to young hydrocephalic rabbits. Only a small number of animals have so far been used in these experiments, but the results are sufficiently consistent as to suggest that they may have some validity.

Three lines of investigation have been followed. In one group, young rabbits diagnosed as hydrocephalic by the presence of an anterior fontanelle were given vitamin A in oil by mouth. In a second group, intraventricular cerebrospinal fluid pressures were recorded by means of a bubble manometer before and after the administration of vitamin A. In a third group, immediately following the initial intraventricular pressure measurement, cerebrospinal fluid was withdrawn, replaced by air and a ventriculogram taken. The subsequent treatment of this group was similar to that of the second group.

The results of these preliminary experiments suggest the following conclusions: the administration of vitamin A to hydrocephalic young rabbits prolongs their survival; the cerebrospinal fluid pressure falls rapidly following treatment with vitamin A; in treated animals which survive for relatively long periods the doming of the calvaria is obscured by the general growth of the skull and apparently also by remodelling of the vault.

Termination of the spino-thalamic tract in man.

By DAVID BOWSHER. *University of Liverpool*

Two human brain stems have been examined by the Nauta method for axon degeneration following unilateral spino-thalamic tractotomy. Fibres have been found to end in the following ipsilateral thalamic nuclei: n. ventralis postero-lateralis, n. centrum medianum, and n. reticularis. Contralaterally, fibres pass by way of the posterior commissure to end in the n. ventralis postero-lateralis.

Other fibres, or their collaterals, of this tract terminate throughout the brain stem reticular formation on both sides. It is suggested that these fibres are eventually projected on to the cortex through the diffuse thalamic reticular system, accounting for (a) central 'slow' and diffuse pain, (b) central reaction to pain, and (c) failure to find specific pain spots on the post-central cortex. The anatomical and surgical implications of these findings are discussed.

Further observations on the nerve endings in the adult dog heart.

By R. L. HOLMES. *University of Leeds*

In a previous communication (Holmes, *Proc. Anat. Soc.*, April 1956) circumscribed nervous endings demonstrated in the atrial wall of the adult dog heart after silver impregnation were described.

Further examination of silver-impregnated material has confirmed the existence of such endings. In addition, the terminal subendothelial nervous network has been demonstrated by silver impregnation in sections cut tangentially through the endocardium. The network was closely associated with a sheet of endocardial smooth muscle, both structures lying within the limits of a 15 μ section.

Study of whole thickness preparations of the atrial wall of the dog heart stained by a methylene-blue technique has shown (a) thick nerve fibres, ending by repeated branching into fine fibres and filaments closely associated with cells, each complex forming a discrete and localized structure; (b) the terminal subendothelial nervous network.

The terminal network is more extensive in distribution and apparently independent of the thicker fibres, and their endings have been seen only in certain regions.

Correlation of the structures demonstrated by silver impregnation with those stained by methylene blue has thus been made. It is considered that true nerve endings, which are probably receptor in function, are present at the terminations of the thick fibres, while the wider distribution of the terminal nervous network might suggest that it fulfils some less specific role.

A neurological study of the cardiac atria of mammals.By T. S. KING and J. B. COAKLEY. *University of Sheffield*

In a series of hearts, from a number of different orders of mammals, the atria have been examined in serial sections, stained by several techniques. The situation and distribution of the nerve cells have been studied and mapped out. The numbers and position of the cells vary from one order to another, but certain features are common to all. The majority of the cells lie in the subepicardial connective tissue, but some lie in the myocardium of the atria and atrial septum. In all the animals ganglia are associated with both nodes and are numerous in the dorsal inter-atrial groove. The different types of cells are described.

A study in the oblique vein of the left atrium and the left precaval vein in mammals. By T. S. KING and J. B. COAKLEY. *University of Sheffield*

The oblique vein of the left atrium has been examined in serial sections in a number of mammalian hearts (guinea-pig, cat, dog, porpoise, ox, sheep, pig, rhesus monkey, human).

It has been traced along the posterior surface of the left atrium to its opening into the coronary sinus. The vein, together with some cardiac muscle fibres, lies in loose subepicardial connective tissue, usually quite separate from the main muscle mass of the atrial wall. In all specimens the vein and muscle are accompanied by nerves and ganglia, the latter being particularly numerous in some hearts.

In a further series of mammalian hearts the left precaval vein has been examined in a similar manner (platypus, wallaroo, hedgehog, mole, rat, rabbits, fruit bat, tree shrew). Only that portion of the vein that lies in relation to the posterior surface of the left atrium has been sectioned. In each animal, in this part of its course it is associated with cardiac muscle, which is arranged as a complete layer in the wall of the vessel. This vein is also accompanied by nerves and ganglia.

Variations of the human intra-abdominal pressure during weight-lifting in different postures. By P. R. DAVIS. *Royal Free Hospital School of Medicine, London.*

During an investigation into the mechanisms of weight carriage by the trunk, consideration has been given to the possibility of extra-vertebral transmission of longitudinal forces. Sir Arthur Keith (1923) found that the intra-abdominal pressure was positive. He noted that in the upright position the intra-rectal pressure exceeded that in the stomach, and that in the horizontal position these two pressures became approximately equal. Other workers have since shown that on straining the gastric and rectal pressures each rise by a similar amount.

These prior findings have been confirmed, and further studies of the intra-abdominal pressure changes have been made during weight-lifting in different postures. In a series of healthy adult males the pressure changes on lifting a weight have followed a common pattern, regardless of posture. This pattern consisted of an abrupt rise in pressure during the lift (the snatch pressure), a rapid fall to a level above the resting pressure while the weight was held in a given position against gravity, and a return to the resting level when the weight was put down. These pressure changes were small in the upright position, greater in the stooping position, and greatest when the body was prone with the legs supported in line with the unsupported trunk. The significance of the findings is discussed.

Persistence of sex chromatin after death in unfixed tissues.By A. D. DIXON and J. B. D. TORR. *University of Manchester*

In a previous communication it was shown that the sex of small fragments of unfixed tissue could be determined from details of nuclear morphology for 2-3 weeks after death, depending on the environment in which the tissue had been allowed to decompose.

In the present study:

(i) The persistence of sex chromatin in unfixed tissues which have been buried in direct contact with the soil was investigated. Small pieces of skin, muscle and cartilage were removed at regular intervals, sectioned, stained with Harris's haematoxylin and eosin and the nuclei examined. Sex chromatin is visible for a longer period than is the case with tissues placed in other environments, being equally well seen in all three types of tissue.

(ii) It was thought that sex chromatin might persist for a longer period in the dental pulp, but in extracted teeth it is not possible to determine the sex by this means for more than 24 hr. due to cell dehydration, and in teeth immersed in normal saline the period of survival does not exceed 1 week.

(iii) Attempts were made to determine sex from an examination of blood on scalpel blades used to obtain post-mortem tissues. Negative results were obtained, due to haemolysis, but sex could be determined from accompanying epithelial cells. A number of blades were kept in room air and sex chromatin could still be demonstrated in this way several weeks later.

(iv) An examination of nuclear morphology in a number of pathological conditions indicated that these and previous findings were applicable both to normal and abnormal tissues.

A comparison of methods for the determination of chromosomal sex and their clinical application. By J. B. D. TORR and A. D. DIXON. *University of Manchester*

We have confirmed the value of the oral smear method for the determination of chromosomal sex. Other workers have demonstrated the use of vaginal smears and blood films in the diagnosis of chromosomal sex and the present investigation has been undertaken (*a*) to compare the three methods, and (*b*) to apply the findings to the determination of chromosomal sex in a series of patients presenting sex abnormalities of an anatomical or psychological nature.

It has been found that the oral smear method is the simplest to perform and the easiest to interpret. Vaginal smears are frequently contaminated with bacteria and masses of amorphous debris and blood films require the examination of a comparatively large number of polymorphonuclear leucocytes in order to ensure an accurate diagnosis.

No difficulty has been experienced in determining the chromosomal sex in the cases of sexual abnormality and the relationship of our results to the clinical picture is discussed.

INDEX TO VOLUME 90

- Abdominal chromaffin tissue, development and fate of, in rabbit. By R. E. Coupland, 527
- Alimentary tract, distribution of 'lipase' in. By B. F. Martin, 440
- Alveolar nerve, middle superior, in man. By M. J. T. Fitzgerald, 520
- Ansa spiralis of ox colon. By R. N. Smith and G. W. Meadows, 523
- Appendix, of rabbit, effects of cortisone on. By E. J. Field, 428
- Artery, aberrant renal. By F. T. Graves, 553
- Ashley, G. T. The relationship between the pattern of ossification and the definitive shape of the mesosternum in man, 87
- Asscher, A. W., Turner, C. H. and de Boer, C. H. Cornification of the human vaginal epithelium, 547
- Auer, J. Terminal degeneration in the diencephalon after ablation of frontal cortex in the cat, 30
- A-v anastomoses in human external ear. By M. M. L. Prichard and P. M. Daniel, 309
- A-v anastomoses, new formation of, in rabbit's ear. By B. Rossatti, 318
- Axon and Schwann cell in peripheral nerve, relation between. By G. Causey and H. Hoffman, 1
- Baddeley, R. M. *See* Eayrs, J. T., joint authors, 161
- Barnett, G. H. A rapid method of graphic reconstruction, 304
- Baxter, J. S. Book review. Embryologie. By D. Starck, 559
- Blood supply of optic nerve and chiasma in man. By E. J. Steele and M. J. Blunt, 486
- Blood supply of rabbit's ear. By B. Rossatti, 318
- Blunt, M. J. and Stratton, Kathleen. The immediate effect of ligature of vasa nervorum, 204
- The development of a compensatory collateral circulation to nerve trunk, 508
- Blunt, M. J. *See* Steele, E. J., joint authors, 486
- Book Reviews:
- Anatomy of the Rat. By E. E. Greene. Reviewed by C. C. D. Shute, 457
- Bibliographie der Menschenaffen. By H. Voss. Reviewed by J. D. Boyd, 159
- Bone. An Introduction to the Physiology of Skeletal Tissue. By F. C. McLean and M. R. Urist. Reviewed by J. C. Brash, 155
- Blood Supply and Anatomy of the Upper Abdominal Organs. By N. A. Michels. Reviewed by G. A. G. Mitchell, 308
- Cardiovascular Innervation. By G. A. G. Mitchell. Reviewed by F. Davies, 457
- Das Zwischenhirn-Hypophysensystem. By W. Bargmann. Reviewed by S. Zuckerman, 559
- Embryologie. By D. Starck. Reviewed by J. S. Baxter, 559
- Growth at Adolescence. By J. M. Tanner. Reviewed by D. A. Sholl, 156
- Handbuch der mikroskopischen Anatomie des Menschen. IV. Band, Nerven system, 2. Teil. By G. Schaltenbrand and E. Dorn. Reviewed by J. W. Millen and D. H. M. Woollam, 159
- Neuro-vascular Hila of Limb Muscles. By J. C. Brash. Reviewed by Ruth E. M. Bowden, 155
- Proctologic Anatomy. By R. V. Gorsch. Reviewed by E. W. Walls, 459
- Radio-anatomie générale de la Tête. By R. Anbanian and J. Porot. Reviewed by J. W. D. Bull, 459
- Segmental Anatomy of the Lungs. By E. A. Boyden. Reviewed by R. J. Harrison, 158
- The Lung: Clinical Physiology and Pulmonary Function Tests. By J. H. Comroe, R. E. Forster, A. B. Dubois, W. A. Briscoe and E. Carlsen. Reviewed by R. J. Harrison, 157
- The Fossil Evidence for Human Evolution. By W. E. Le Gros Clark. Reviewed by J. D. Boyd, 307
- Bowden, Ruth E. M. Book review. Neuro-vascular Hila of Limb Muscles. By J. C. Brash, 155
- Bowden, Ruth E. M. and Mahran, Z. Y. The functional significance of the pattern of innervation of the muscle quadratus labii superioris of the rabbit, cat and rat, 217
- Boyd, J. D. Book review. Bibliographie der Menschenaffen. By H. Voss, 159
- Book Review. The Fossil Evidence for Human Evolution. By W. E. Le Gros Clark, 307
- Brash, J. C. Book review. Bone. An Introduction to the Physiology of Skeletal Tissue. By F. C. McLean and M. R. Urist, 155
- Britton, W. A. *See* Tomasch, J., joint authors, 337
- Brodal, A. *See* Rossi, G. F., joint authors, 42
- Buchanan, T. J. *See* Walls, E. W., joint authors, 329
- Bull, J. W. D. Book review. Radio-anatomie générale de la Tête. By R. Anbanian and J. Porot, 459
- Bulmer, D. The early stages of vaginal development in the sheep, 123
- Bunn, D. I. G. and Turner, P. The representation of skull shape by contour drawing, 298
- Capillaries of human nail fold. By E. W. Walls and T. J. Buchanan, 329
- Causey, G. and Hoffmann, H. The relation between the Schwann cell and the axon in peripheral nerves, 1
- The ultrastructure of the synaptic area in the superior cervical ganglion, 502
- Cerebellar vermal projections in brain stem of cat. By D. M. Thomas *et al.*, 371

- Chambers, W. M. *See* Thomas, D. M., joint authors, 371
- Chromaffin tissue, abdominal, development and fate of, in rabbit. By R. E. Coupland, 527
- Circulation in spleen, rabbit, development of. By O. J. Lewis, 282
- Collateral circulation, development of compensatory, to nerve trunk. By M. J. Blunt and K. Stratton, 508
- Colon, ox, ansa spiralis of. By R. N. Smith and G. W. Meadows, 523
- Connective tissue, adult mouse, uptake of radioactive sulphate in. By A. Glücksmann, A. Howard and S. R. Pelc, 478
- Contour drawing, representation of skull shape by. By D. I. G. Bunn and P. Turner, 298
- Cornification of human vaginal epithelium. By A. W. Asscher, C. J. Turner and C. H. de Boer, 547
- Cortex, cerebral, guinea-pig, development of. By A. Hughes and L. B. Flexner, 386
- Cortico-reticular fibres, in cat. By G. F. Rossi and A. Brodal, 42
- Cortisone acetate and response of regional lymph node to skin homograft. By R. J. Scothorne, 417
- Cortisone, morphological effects of, in rabbit. By E. J. Field, 428
- Coupland, Rex E. The development and fate of the abdominal chromaffin tissue in the rabbit, 527
- Cowan, W. M. and Powell, T. P. S. A note on terminal degeneration in the hypothalamus, 188
- Cricetus auratus*, early development of. By W. J. Hamilton and D. M. Samuel, 395
- Daniel, P. M. *See* Prichard, M. M. L., joint authors, 309
- Davies, D. Book review. Cardiovascular Innervation. By G. A. G. Mitchell, 457
- Davies, J. Histochemistry of the rabbit placenta, 135
- de Boer, C. H. *See* Asscher, A. W., joint authors, 547
- Development, early, of hamster. By W. J. Hamilton and D. M. Samuel, 395
- Dickson, A. D. The ductus venosus of the pig, 143
- Diencephalon, terminal degeneration in, after frontal ablation. By J. Auer, 30
- Duckworth, Dr W. L. H. *In memoriam* notice by F. Goldby, 455
- Ductus venosus, of pig. By A. D. Dickson, 143
- Ear, human external, a-v anastomoses in. By M. M. L. Prichard and P. M. Daniel, 309
- Ear, rabbit, blood supply of. By B. Rosatti, 318
- Eardrum, mammalian, evolution of. By C. C. D. Shute, 261
- Eayrs, J. T. and Baddeley, R. M. Neural pathways in lactation, 161
- Emery, L. J. The distribution of haemopoietic foci in the infantile human liver, 293
- Epithelium, transitional, and osteogenesis. By F. R. Johnson and R. M. H. McMinn, 106
- Epithelium, vaginal, human, cornification of. By A. W. Asscher, C. F. Turner and C. H. de Boer, 547
- Evans, D. H. L. and Hamlyn, L. H. A study of silver degeneration methods in the central nervous system, 193
- Evolution of mammalian eardrum and tympanic cavity. By C. C. D. Shute, 261
- Fearnhead, R. W. and Linder, J. E. Observations on the silver impregnation of nerve fibres in teeth, 228
- Field, E. J. Some morphological effects of large doses of cortisone in the rabbit with special reference to the thymus and appendix, 428
- Fishes, growth changes in peripheral nerve fibres of. By P. K. Thomas, 5
- Fitzgerald, M. J. T. The occurrence of a middle superior alveolar nerve in man, 520
- Flexner, L. B. *See* Hughes, A., joint authors, 386
- Ford, E. H. R. The growth of the foetal skull, 63
- Fornix, post-commissural, of rat, degeneration in. By R. W. Guillery, 350
- Gamble, H. J. An experimental study of the secondary olfactory connexions in *Testudo graeca*, 15
- Girgis, F. G. *See* Pritchard, J. J., joint authors, 73
- Glenister, T. W. The development of the penile urethra in the pig, 461
- Glücksmann, A., Howard, Alma and Pelc, S. R. The uptake of radioactive sulphate by cells, fibres and ground-substance of mature and developing connective tissue in the adult mouse, 478
- Graves, F. T. The aberrant renal artery, 553
- Growth of foetal skull. By E. H. R. Ford, 63
- Guillery, R. W. Degeneration in the post-commissural fornix and the mamillary peduncle of the rat, 350
- Haddara, M. A quantitative study of the post-natal changes in the packing density of the neurons in the visual cortex of the mouse, 494
- Haemopoietic foci in infantile human liver. By J. L. Emery, 293
- Hamilton, W. J. and Samuel, D. M. (the late). The early development of the golden hamster (*Cricetus auratus*), 395
- Hamlyn, L. H. *See* Evans, D. H. L., joint authors, 193
- Hamster, golden, early development of. By W. J. Hamilton and D. M. Samuel, 395
- Harrison, R. J. Book review. Segmental Anatomy of the Lungs. By E. A. Boyden, 158
- Book review. The Lung: Clinical Physiology and Pulmonary Function Tests. By J. H. Comroe *et al.*, 157
- Histochemistry of rabbit placenta. By J. Davies, 135
- Hoffman, H. *See* Causey, G., joint authors, 502
- Howard, Alma. *See* Glücksmann, A., joint authors, 478
- Hughes, A. and Flexner, L. B. A study of the development of the cerebral cortex of the foetal guinea-pig by means of the ultra-violet microscope, 386

- Hypothalamus, terminal degeneration in. By W. M. Cowan and T. P. S. Powell, 188
- In Memoriam:
Duckworth, W. L. H., 455
- Innervation of *quadratus labii superioris*. By Ruth E. M. Bowden and Z. Y. Mahran, 217
- Jacobson's organ. By V. E. Negus, 515
- Johnson, F. R. and McMinn, R. M. H. Transitional epithelium and osteogenesis, 106
- Kaufman, R. K. See Thomas, D. M., joint authors, 371
- Knee joint, human, postural mechanism of. By J. W. Smith, 236
- Kynaston Thomas, P. Growth changes in the diameter of peripheral nerve fibres in fishes, 5
- Lactation, neural pathways in. By J. T. Eayrs and R. M. Baddeley, 161
- Levator palati muscle. By R. F. Rohan and L. Turner, 153
- Lewis, O. J. The development of the circulation in the spleen of the foetal rabbit, 282
- Linder, J. E. See Fearnhead, R. W., joint authors, 228
- 'Lipase', distribution of in alimentary tract. By B. F. Martin, 440
- Liver, infantile human, distribution of haemopoietic foci in. By J. L. Emery, 293
- Lymph node, regional, effect of cortisone on response of. By R. J. Scothorne, 417
- Mahran, Z. Y. See Bowden, Ruth E. M., joint authors, 217
- Maxillary peduncle, of rat, degeneration in. By R. W. Guillery, 350
- Martin, B. F. The histochemical distribution of 'lipase' in the alimentary tract and associated glands of laboratory animals, 440
- McMinn, R. M. H. See Johnson, F. R., joint authors, 106
- Meadows, G. W. See Smith, R. N., joint authors, 523
- Mesosternum, in man, ossification and shape of. By G. T. Ashley, 87
- Methods, degeneration, silver, in C.N.S. By D. H. L. Evans and L. H. Hamlyn, 193
- Millen, J. W. Book review. Nervensystem. By G. Schaltenbrand and E. Dorn, 159
- Mitchell, G. A. G. Book review. Blood supply and Anatomy of the Upper Abdominal Organs. By N. A. Michels, 308
- Morphological effects of cortisone in rabbit. By E. J. Field, 428
- Muscle, levator palati. By R. F. Rohan and L. Turner, 153
- Muscle *quadratus labii superioris*, pattern of innervation of. By Ruth E. M. Bowden and Z. Y. Mahran, 217
- Myelinated fibre populations. By T. A. Quilliam, 172
- Nail fold, human, capillary blood vessels of. By E. W. Walls and T. J. Buchanan, 329
- Negus, V. E. The organ of Jacobson, 515
- Nerve, alveolar, middle superior, in man. By M. J. T. Fitzgerald, 520
- Nerve fibres in teeth, silver impregnation of. By R. W. Fearnhead and J. E. Linder, 228
- Nerve fibres, peripheral in fishes, growth changes in diameter of. By P. K. Thomas, 5
- Nerve trunk, development of compensatory collateral circulation to. By M. J. Blunt and K. Stratton, 508
- Nerves, human peripheral, individual variability of fibre composition in. By J. Tomasch and W. A. Britton, 337
- Nerves, peripheral, relation between axon and Schwann cells in. By G. Causey and H. Hoffman, 1
- Neural pathways in lactation. By J. T. Eayrs and R. M. Baddeley, 161
- Neurons in visual cortex of mouse. By M. Haddara, 494
- Olfactory connexions, secondary, in *Testudo graeca*. By H. J. Gamble, 15
- Optic nerve and chiasma, in man, blood supply of. By E. J. Steele and M. J. Blunt, 486
- Organ of Jacobson. By V. E. Negus, 515
- Osteogenesis, and transitional epithelium. By F. R. Johnson and R. M. H. McMinn, 106
- Pelc, S. R. See Glücksmann, A., joint authors, 478
- Peritoneum, role of, in formation of septum recto-vesicale. By P. H. S. Silver, 538
- Phosphatase, alkaline, in developing teeth, of rat. By N. B. B. Symons, 117
- Pig, development of penile urethra in. By T. W. Glenister, 461
- Pig, ductus venosus of. By A. D. Dickson, 143
- Placenta, rabbit, histochemistry of. By J. Davies, 135
- Powell, T. P. S. See Cowan, W. M., joint authors, 188
- Prichard, M. M. L. and Daniel, P. M. Arterio-venous anastomoses in the human external ear, 309
- Pritchard, J. J., Scott, J. H. and Girgis, F. G. The structure and development of cranial and facial sutures, 73
- Quilliam, T. A. Some characteristics of myelinated fibre populations, 172
- Rabbit, abdominal chromaffin tissue in. By R. E. Coupland, 527
- Reconstruction, graphic, rapid method of. By C. H. Barnett, 304
- Renal artery, aberrant. By F. T. Graves, 553
- Renal glomerular efferent vessel. By J. P. Smith, 290
- Ribonucleic acid in developing teeth, of rat. By N. B. B. Symons, 117
- Rohan, R. F. and Turner, L. The levator palati muscle, 153
- Rossatti, B. Observations on the blood supply of the rabbit's ear and on the experimental new formation of arterio-venous anastomoses, 318
- Rossi, G. F. and Brodal, A. Corticofugal fibres to the brain-stem reticular formation. An experimental study in the cat, 42

- Samuel, D. M. (the late). *See* Hamilton, W. J., joint authors, 395
- Schwann cell and axon in peripheral nerves, relation between. By G. Causey and H. Hoffman, 1
- Scothorne, R. J. The effect of cortisone acetate on the response of the regional lymph node to a skin homograft, 417
- Scott, J. H. *See* Pritchard, J. J., joint authors, 73
- Septum recto-vesicale, role of peritoneum in formation of. By P. H. S. Silver, 538
- Sheep, early stages of vaginal development in. By D. Bulmer, 123
- Sholl, D. A. Book review. Growth at Adolescence. By J. M. Tanner, 156
- Shute, C. C. D. The evolution of the mammalian ear-drum and tympanic cavity, 261
- Book review. Anatomy of the Rat. By E. E. Greene, 457
- Silver, P. H. S. The role of the peritoneum in the formation of the septum recto-vesicale, 538
- Skin homograft, effect of cortisone on responses of lymph node to. By R. J. Scotthorne, 417
- Skull, foetal, growth of. By E. H. R. Ford, 63
- Skull shape, representation of, by contour drawing. By D. I. G. Bunn and P. Turner, 298
- Smith, J. P. Anatomical features of the human renal glomerular efferent vessel, 290
- Smith, J. W. Observations on the postural mechanism of the human knee joint, 236
- Smith, R. N. and Meadows, G. W. The arrangement of the ansa spiralis of the ox colon, 523
- Spleen, rabbit, development of circulation in. By O. J. Lewis, 282
- Sprague, J. M. *See* Thomas, D. M., joint authors, 371
- Steele, E. J. and Blunt, M. J. The blood supply of the optic nerve and chiasma in man, 486
- Stratton, Kathleen. *See* Blunt, M. J., joint authors, 204, 508
- Sutures, cranial and facial, structure and growth of. By J. J. Pritchard, J. H. Scott and F. G. Girgis, 73
- Symons, N. B. B. Ribonucleic acid-alkaline phosphatase distribution in the developing teeth of the rat, 117
- Synaptic area in superior cervical ganglion, ultra structure of. By G. Causey and H. Hoffman, 502
- Teeth, developing, of rat, ribonucleic acid-alkaline phosphatase distribution in. By N. B. B. Symons, 117
- Teeth, silver impregnation of nerve fibres in. By R. W. Fearnhead and J. E. Linder, 228
- Testudo graeca*, secondary olfactory connexions in. By H. J. Gamble, 15
- Thomas, D. M., Kaufman, R. K., Sprague, J. M. and Chambers, W. M. Experimental studies of the vermal cerebellar projections in the brain stem of the cat (fastigiobulbar tract), 371
- Thymus, of rabbit, effects of cortisone on. By E. J. Field, 428
- Tomasch, J. and Britton, W. A. On the individual variability of fibre composition in human peripheral nerves, 337
- Tract, fastigiobulbar, in cat. By D. M. Thomas *et al.*, 371
- Turner, C. J. *See* Asscher, A. W., joint authors, 547
- Turner, L. *See* Rohan, R. F., joint authors, 153
- Turner, P. *See* Bunn, D. I. G., joint authors, 298
- Tympanic cavity, mammalian, evolution of. By C. C. D. Shute, 261
- Ultrastructure of synaptic area in superior cervical ganglion. By G. Causey and H. Hoffman, 502
- Ultra-violet microscopy and development of cerebral cortex. By A. Hughes and L. B. Flexner, 386
- Urethra, penile, development of, in pig. By T. W. Glenister, 461
- Vagina, in sheep, early stage of development of. By D. Bulmer, 123
- Vaginal epithelium, human, cornification of. By A. W. Asscher, C. J. Turner and C. H. de Boer, 547
- Vasa nervorum, immediate effects of ligature of. By M. J. Blunt and K. Stratton, 204
- Visual cortex of mouse, packing of neurons in. By M. Haddara, 494
- Walls, E. W. Book review. Proctologic Anatomy. By R. V. Gorsch, 459
- Walls, E. W. and Buchanan, T. J. Observations on the capillary blood vessels of the human nail fold, 329
- Woollam, D. H. M. *See* Millen, J. W. joint reviewer, 159
- Zuckerman, S. Book review. Das Zwischenhirn-Hypophysensystem. By W. Bargmann, 559

SUPPLEMENTARY INDEX OF PROCEEDINGS

- Adams, D. J. *See* Braithwaite, J. L., joint authors, 596
- Adrenal medulla, rat, electron microscope appearances of. By J. D. Lever, 569
- Adreno-cortical mitochondria, fine structure of. By J. D. Lever, 589
- Allbrook, D. B. and Chagula, W. K. Theoretical basis for segmental variations, 586
- Alveolar nerve, middle superior, in man. By M. J. T. Fitzgerald, 586
- Anal canal, epithelium of adult. By E. W. Walls and R. P. Gould, 561
- Anal mucosa, in mice, results of resection of. By R. J. O'Connor, 570
- Aortic nerve of horse. By A. S. King, 571
- Armstrong, J. A. Investigation of tissue fluorescence induced by acridine orange, 598
- Arteries, of hind-brain, development of, in rat. By D. B. Moffat, 585
- Arteries, spiral, of pregnant uterus, cells in. By J. D. Boyd and W. J. Hamilton, 595
- Artery, aberrant renal. By F. T. Graves, 587
- Ashley, G. T. A comparison of human and anthropoid mesosterna, 587
- Asscher, A. W., Turner, C. J. and de Boer, C. H. Sulphydryl and disulphide groups in the human vaginal epithelium, 577
- Atria, cardiac, of mammals, neurological study of. By T. S. King and J. B. Coakley, 601
- Autoradiography in guinea-pig tissues. By C. Ruth Hill and G. H. Bourne, 576
- Backhouse, K. M. and Butler, H. The development of the human gubernaculum testis, 584
- Bacsich, P. and Wyburn, G. M. Fate and behaviour of the second set of subcutaneous corneal homografts in the guinea-pig, 583
- Bacsich, P. *See* Wyburn, G. M., joint authors, 563
- Badger, delayed implantation in. By R. J. Harrison, E. G. Neal and C. J. Turner, 574
- Barnett, C. H. A rapid method of graphic reconstruction, 564
- Batten, E. H. The activity of the placodes during the development of the mixed cranial nerves in the sheep embryo, 585
- Baxter, J. S. Intravital staining of the rat testis with trypan blue, 596
- Beckett, Evelyn B. and Bourne, G. H. Some observations on the histochemistry of goat foetal cardiac and skeletal muscle, 576
- Bellairs, A. d'A. *See* Crompton, A. W., joint authors, 571
- Blood supply of optic nerve and chiasma. By E. J. Steele and M. J. Blunt, 564
- Blunt, M. J. *See* Steele, E. J., joint authors, 564
- Bond induction by callus and cartilage in rabbit. By J. B. Bridges and J. J. Pritchard, 563
- Bone growth, histological study of, using Alizarin. By D. A. N. Hoyte, 585
- Bone induction in renal ischaemia. By J. B. Bridges, 595
- Bone repair in salamander. By J. Bowden and J. J. Pritchard, 572
- Bones, microradiology of blood supply of. By M. Brookes and R. G. Harrison, 564
- Bonner, W. N. Delayed implantation in the southern elephant seal, 574
- Bony cuff of mammals, fate and significance of. By C. W. M. Pratt, 596
- Bourne, G. H. *See* Beckett, Evelyn B., joint authors, 576
- *See* Hill, C. Ruth, joint authors, 576
- Some histochemical observations on Purkinje cells, 576
- Boutons terminaux*, of cat, size in sixth cervical segment. By G. W. Pearce, 556
- Bowden, J. The fine structure of lamellibranch muscle, 594
- Bowden, J. and Pritchard, J. J. Bone repair in the salamander, 572
- Bowsher, D. Early changes in the mouse spinal cord following irradiation with focussed ultrasound, 579
- Termination of the spino-thalamic tract in man, 600
- Boyd, J. D. The pharyngeal hypophysis, 595
- Boyd, J. D. and Hamilton, W. J. Cells in the spiral arteries of the pregnant uterus, 595
- Brain, Sir Russell. Congenital abnormalities of the cervical vertebrae, 579
- Braithwaite, J. L. and Adams, D. J. Splenic venography in the rat, 596
- Brandes, D. Histochemical demonstration of alkaline and acid phosphatases in electron micrographs, 578
- Bridges, J. B. Bone induction in experimental renal ischaemia, 595
- Bridges, J. B. and Pritchard, J. J. Bone induction by implants of callus, cartilage and other tissues in rabbit, 563
- Induction of cartilage and bone by devitalized implants under the kidney capsule of rabbits, 593
- Brookes, M. and Harrison, R. G. Microradiology of the blood supply of long bones, 564
- Bulmer, D. Some aspects of vaginal development, 570
- Bunn, D. I. G. and Turner, P. Contour reconstruction of skull, 562
- Butler, H. *See* Backhouse, K. M., joint authors, 584
- Cartilage homografts, uptake of ^{35}S in. By G. M. Wyburn and P. Bacsich, 563
- Cartilage homografts, use of ^{35}S in. By P. A. Ring, 582
- Cauna, N. Cytological studies of digital touch corpuscles, 567
- Cauna, N. and Mannan, G. Function of digital Pacinian corpuscles, 588
- Cervical vertebrae, congenital abnormalities of. By Sir Russell Brain, 579

- Chagula, W. K. *See* Allbrook, D. B., joint authors, 586
- Choroid plexus epithelium in rabbit, fine structure of. By J. W. Millen and G. E. Rogers, 589
- Chromaffin tissue, extra-adrenal, post-natal fate of, in rodents. By R. E. Coupland, 597
- Chromosomal sex, post-mortem morphology of. By A. D. Dixon and J. B. D. Torr, 582
- Clegg, E. J. The vascular arrangements within the human prostate gland, 580
- Coakley, J. B. *See* King, T. S., joint authors, 601
- Conjunctiva, encapsulated nerve endings in. By D. R. Oppenheimer, Elizabeth Palmer and A. G. M. Weddell, 579
- Corneal homografts, fate of. By P. Bacsich and G. M. Wyburn, 583
- Coupland, R. E. The post-natal fate of the abdominal extra-adrenal chromaffin tissue in rodents, 597
- Cowan, W. M., Powell, T. P. S. and Guillery, R. W. Quantitative study of fornix-mamillo-thalamic system, 590
- Cowan, W. M. *See* Powell, T. P. S., joint authors, 566
- Cragg, B. G. and Hamlyn, L. H. Histological study of commissural and septal connexions of hippocampus in rabbit, 591
- Commissural and septal connexions of the hippocampus in rabbit, 591
- Cranial base, growth of. By J. H. Scott, 594
- Crompton, A. W. and Bellairs, A. d'A. Ictidosaurs: a link between reptiles and mammals, 571
- C.-s.-f. pressure and hydrocephalus in rabbits. By J. W. Millen and D. H. M. Woollam, 567
- Cyclops fetuses, two human. By P. H. S. Silver, 561
- Davis, P. R. Variations of intra-abdominal pressure during weight-lifting in different postures, 601
- de Boer, C. H. *See* Asscher, A. W., joint authors, 577
- Dentine, human, innervation of. By R. W. Fearnhead, 575
- Dick, D. A. T. Development of hematic cell in foetal sheep, 568
- Dickson, A. D. and Millen, J. W. Glycogen body of chick spinal cord, 589
- Dickson, A. D. *See* Millen, J. W., joint authors, 599
- Dixon, A. D. and Torr, J. B. D. Persistence of sex chromatin after death in unfixed tissues, 601
- Post-mortem morphology of chromosomal sex, 582
- Dixon, A. D. *See* Torr, J. B. D., joint authors, 581, 602
- Duplication of stomatodaeal structures in infant. By W. R. M. Morton, 594
- Ear-drum, mammalian evolution of. By C. C. D. Shute, 572
- Electron micrographs, demonstration of phosphatases in. By D. Brandes, 578
- Electron microscope observations on hairs and hair follicles. By G. E. Rogers, 569
- Electron microscopy of rat adrenal medulla. By J. D. Lever, 569
- Epithelium, human odontogenic and adjacent oral, remnants of. By C. H. Tonge, 584
- Epithelium of adult anal canal. By E. W. Walls and R. P. Gould, 561
- Epithelium, transitional, alkaline phosphatase in. By B. F. Martin, 577
- Evolution of mammalian ear-drum and tympanic cavity. By C. C. D. Shute, 572
- Evolution of parotid gland and its secretomotor innervation. By C. C. D. Shute, 580
- Extra-dural venous system, in mammals. By J. W. D. Tomlinson, 575
- Fearnhead, R. W. Histological evidence for innervation of human dentine, 575
- Fitzgerald, M. J. T. Occurrence of middle superior alveolar nerve in man, 586
- Fluorescence, tissue, induced by acridine orange. By J. A. Armstrong, 589
- Ford, E. H. R. Growth-rates in foetal skull, 562
- Fornix-mamillo-thalamic system, quantitative study of. By W. M. Cowan, T. P. S. Powell and R. W. Guillery, 590
- Fornix, post-commissural, non-mamillary fibres in. By R. W. Guillery, 565
- Gall bladder, of cat, epithelialization of mucosal lesions in. By R. M. H. McMinn and F. R. Johnson, 578
- Girgis, F. G. and Pritchard, J. J. Cartilage in repair of skull vault, 573
- Experimental alteration of cranial suture patterns, 573
- Glees, P. *See* Pearce, G. W., joint authors, 599
- Glees technique, use of in hypothalamus and preoptic regions. By T. P. S. Powell and W. M. Cowan, 566
- Glenister, T. W. Development of prepuce in man, 582
- Gould, R. P. *See* Walls, E. W., joint authors, 561
- Graves, F. T. The aberrant renal artery, 587
- Growth of cranial base. By J. H. Scott, 594
- Gubernaculum testis, human, development of. By K. M. Backhouse and H. Butler, 584
- Guillery, R. W. Non-mamillary fibres in post-commissural fornix, 565
- *See* Cowan, W. M., joint authors, 590
- Gut, of rat, distribution of extrinsic nerve fibres in. By G. Schofield, 592
- Hair follicles, formation of hair germ in. By W. Montagna, 575
- Hairs and hair follicles, electron microscopy of. By G. E. Rogers, 569
- Hamilton, W. J. *See* Boyd, J. D., joint authors, 595
- Hamlyn, L. H. *See* Cragg, B. G., joint authors, 591
- Harrison, R. G. *See* Brookes, M., joint authors, 564
- Harrison, R. J., Neal, E. G. and Turner, C. J. Delayed implantation in badger, 574
- Harrison, R. J. *See* Montagna, W., joint authors, 597
- Harrison, T. J. Effects of hindlimb amputation on the rat pelvis, 563

- Harrison. Sacral growth in rat, 594
- Heart, dog, nerve endings in. By R. L. Holmes, 600
- Heart, dog, receptor nerve endings in. By R. L. Holmes, 588
- Hepatic cell, development of in foetal sheep. By D. A. T. Dick, 568
- Hill, C. Ruth and Bourne, G. H. Autoradiographic studies of uptake of ^{35}S in normal and scorbutic guinea-pig tissues, 576
- Hippocampus, rabbit, commissural and septal connexions in. By B. G. Cragg and L. H. Hamlyn, 591
- Hippocampus, rabbit, histology of connexions of. By B. G. Cragg and L. H. Hamlyn, 591
- Holmes, R. L. Further observations on nerve endings in adult dog heart, 600
- Iris reactions to foetal pineal homografts, 567
- Receptor nerve endings in adult dog heart, 588
- Homografts, cartilage, uptake of ^{35}S in. By G. M. Wyburn and P. Bacsich, 563
- Homografts, cartilage, use of ^{35}S in. By P. A. Ring, 582
- Hoyte, D. A. N. Study of bone growth using Alizarin Red A.S., 585
- Hughes, A. Development of spinal cord of *Xenopus*, 590
- Hughes, H. Film of parturition in fruit-bat, 505
- Hydrocephalus, in rabbits, treatment of. By J. W. Millen and A. D. Dickson, 599
- Hyena, spotted, placentation in. By W. R. M. Morton, 581
- Hypophysis, pharyngeal. By J. D. Boyd, 595
- Ictidosaur, link between reptiles and mammals. By A. W. Crompton and A. d'A. Bellairs, 571
- Implantation, delayed, in badger. By R. J. Harrison, E. G. Neal and C. J. Turner, 574
- Implantation, delayed, in seal. By W. N. Bonner, 574
- Implants, devitalized, induction of bone and cartilage by. By J. B. Bridges and J. J. Pritchard, 593
- Innervation, secreto-motor, of parotid gland, evolution of. By C. C. D. Shute, 580
- Johnson, F. R. *See* McMinn, R. M. H., joint authors, 578
- King, A. S. Upper cervical course of aortic nerve of horse, 571
- King, T. S. and Coakley, J. B. Neurological study of the cardiac atria of mammals, 601
- Oblique vein of left atrium and left precaval vein in mammals, 601
- Knee joint, geometry and kinematics of. By C. C. D. Shute, 586
- Lee, J. and Ring, P. A. Effect of maternally administered cortisone upon pancreas of foetus, 583
- Leprosy as aid in investigation of neuro-histology of skin. By G. Weddell, Elizabeth Palmer and G. C. Schofield, 599
- Lever, J. D. Electron microscope appearances of rat adrenal medulla, 569
- Lever, J. D. Fine structure of adreno-cortical mitochondria, 589
- Lewis, O. J. Development of circulation in spleen of foetal rabbit, 569
- Logie, D. *See* McKenzie, J., joint authors, 598
- Lymphocytes, of guinea-pig, in thoracic duct lymph and bone marrow. By V. B. Pathak, W. O. Reinhardt and J. M. Yoffey, 568
- McKenzie, J. Abnormalities in human dicephalic tetrabrachiate monster, 587
- McKenzie, J., Philip, J. F. and Logie, D. Effect of radon exposure on developing maxilla, 598
- Mackinnon, P. C. B. Emotional sweating and suprarenal cortex, 562
- McMinn, R. M. H. and Johnson, F. R. Epithelialization of mucosal lesions in the gall bladder of cat, 578
- Mannan, G. *See* Cauna, N., joint authors, 588
- Martin, B. F. Histochemical localization of alkaline phosphatase in transitional epithelium and its relationship to fatty material, 577
- Mucus-secreting cells of alimentary tract and associated glands, 597
- Maturana, H. Differences in regenerative power of afferent and efferent fibres in optic nerve of anurans, 592
- Melanocytes, tyrosinase in. By G. Szabo, 593
- Mesosterna, human and anthropoid. By G. T. Ashley, 587
- Microradiography and X-ray microscopy, advances in. By G. A. G. Mitchell, 598
- Millen, J. W. and Dickson, A. D. Treatment of hydrocephalus, in rabbits, due to maternal hypovitaminosis A, 599
- Millen, J. W. *See* Dickson, A. D., joint authors, 589
- Millen, J. W. and Rogers, G. E. Fine structure of chorioidal epithelium in rabbit, 589
- Millen, J. W. and Woollam, D. H. M. Relationship between cerebro-spinal fluid pressure and occurrence of hydrocephalus in young of female rabbits subjected to experimental hypovitaminosis A, 567
- Mitchell, G. A. G. Advances in microradiography and X-ray microscopy, 598
- Mitochondria, adreno-cortical, fine structure of. By J. D. Lever, 589
- Mitosis, premeiotic, in mammals. By P. V. Tobias, 570
- Moffat, D. B. Development of hind-brain arteries in rat, 585
- Monster, human, dicephalic tetrabrachiate. By J. McKenzie, 587
- Montagna, W. Formation of hair germ in hair follicles, 575
- Montagna, W. and Harrison, R. J. Adaptations in skin of seal, 597
- Morton, W. R. M. Duplication of stomatodaeal structures in infant, 594
- Placentation in spotted hyena, 581
- Muscle, goat foetal cardiac and skeletal, histochemistry of. By Evelyn B. Beckett and G. H. Bourne, 576
- Muscle, lamellibranch, fine structure of. By J. Bowden, 594

- Mucus-secreting cells of alimentary tract. By B. F. Martin, 597
- Nasopharynx, bilateral fistula of. By C. P. Wilson, 579
- Neal, E. G. *See* Harrison, R. J., joint authors, 574
- Negus, V. E. Organ of Jacobson, 571
- Nerve endings, encapsulated, in conjunctiva. By D. R. Oppenheimer, Elizabeth Palmer and A. G. M. Weddell, 579
- Nerve fibres, extrinsic, distribution of, in rat. By G. Schofield, 592
- Neolithic remains. By L. H. Wells, 580
- O'Connor, R. J. Results of resecting anal mucosa in mice, 570
- Oppenheimer, D. R., Palmer, Elizabeth and Weddell, A. G. M. Encapsulated nerve endings in conjunctiva, 579
- Optic nerve and chiasma, blood supply of. By E. J. Steele and M. J. Blunt, 564
- Optic nerve of anurans, regeneration in. By H. Maturana, 592
- Organ of Jacobson. By V. E. Negus, 571
- Ossification patterns in twin and triplet sheep fetuses. By R. N. Smith, 581
- Pacinian corpuscles, digital. By N. Cauna and G. Mannan, 588
- Palmer, Elizabeth. *See* Oppenheimer, D. R., joint authors, 579
- *See* Weddell, G., joint authors, 599
- Pancreas, foetal, effect of maternally administered cortisone upon. By J. Lee and P. A. Ring, 583
- Parotid gland, evolution of. By C. C. D. Shute, 580
- Parturition in fruit bat, film of. By H. Hughes, 505
- Pathak, V. B., Reinhardt, W. O. and Yoffey, J. M. Lymphocytes in thoracic duct lymph and bone marrow of guinea-pig, 568
- Pearce, G. W. Size of *boutons terminaux* in sixth cervical segment of cat, 566
- Termination of crossed tecto-spinal tract in cat, 565
- Pearce, G. W. and Glees, P. Tectal projection to reticular formation in cat, 599
- Pelvis, rat, effect of hindlimb amputation on. By T. J. Harrison, 563
- Pharyngeal hypophysis. By J. D. Boyd, 595
- Philip, J. F. *See* McKenzie, J., joint authors, 598
- Pineal homografts, foetal, iris reactions to. By R. L. Holmes, 567
- Placentation in spotted hyena. By W. R. M. Morton, 581
- Placodes, activity of in sheep embryos. By E. H. Batten, 585
- Powell, T. P. S. and Cowan, W. M. Glees technique in hypothalamus and preoptic regions, 566
- Powell, T. P. S. *See* Cowan, W. M., joint authors, 590
- Pratt, C. W. M. Fate and significance of primary bony cuff of mammalian long bone, 596
- Prepuce, in man, development of. By T. W. Glenister, 582
- Pressure, intra-abdominal, during weight-lifting. By P. R. Davis, 601
- Pritchard, J. J. *See* Bowden, J., joint authors, 572
- *See* Bridges, J. B., joint authors, 563, 593
- *See* Girgis, F. G., joint authors, 573
- Prostate gland, human, vascular arrangements within. By E. J. Clegg, 580
- Purkinje cells, histochemical observations on. By G. H. Bourne, 576
- Radon, effect of on developing maxilla. By J. McKenzie, J. F. Philip and D. Logie, 598
- Reconstruction, contour, of skull. By D. I. G. Bunn and P. Turner, 562
- Reconstruction, graphic. By C. H. Barnett, 564
- Reinhardt, W. O. *See* Pathak, V. B., joint authors, 568
- Reticular formation, tectal projection to, in cat. By G. W. Pearce and P. Glees, 599
- Ring, P. A. Use of ^{35}S in cartilage homografts, 582
- *See* Lee, J., joint authors, 583
- Rogers, G. E. Electron microscope observations on hairs and hair follicles, 569
- *See* Millen, J. W., joint authors, 589
- Sacral growth in rat. By T. J. Harrison, 594
- Schofield, G. Distribution of extrinsic nerve fibres in gut of rat, 592
- Schofield, G. C. *See* Weddell, G., joint authors, 599
- Scott, J. H. Growth of cranial base, 594
- Seal, delayed implantation in. By W. N. Bonner, 574
- Segmental variations, theoretical basis for. By D. B. Allbrook and W. K. Chagula, 586
- Sex chromatin, persistence of, after death. By A. D. Dixon and J. B. D. Torr, 601
- Sex, chromosomal, determination of, clinical applications. By J. B. D. Torr and A. D. Dixon, 582
- Sex, chromosomal, oral smear determination of. By J. B. D. Torr and A. D. Dixon, 581
- Shute, C. C. D. Evolution of mammalian eardrum and tympanic cavity, 572
- Evolution of parotid gland and its secretomotor innervation, 580
- Geometry and kinematics of knee joint, 586
- Silver, P. H. S. Two human cyclops fetuses, 561
- Skin of seal, adaptations in. By W. Montagna and R. J. Harrison, 597
- Skull, foetal, growth-rates in. By E. H. R. Ford, 562
- Skull vault, cartilage in repair of. By F. G. Girgis and J. J. Pritchard, 573
- Smith, R. N. Ossification patterns in twin and triplet sheep fetuses, 581
- Spinal cord, chick, glycogen body of. By A. D. Dickson and J. W. Millen, 589
- Spinal cord, development of in *Xenopus*. By A. Hughes, 590
- Spinal cord, localization of excitation and inhibition in. By J. M. Sprague, 590
- Spinal cord, mouse, changes following irradiation with focused ultra sound. By D. Bowsher, 579
- Spleen, foetal rabbit, development of circulation in. By O. J. Lewis, 569

- Sprague, J. M. Localization of excitation and inhibition in spinal cord, 590
- Steele, E. J. and Blunt, M. J. Blood supply of optic nerve and chiasma, 564
- Stomatodaeal structures, duplication of. By W. R. M. Morton, 594
- Suprarenal cortex, emotional sweating and. By P. C. B. Mackinnon, 562
- Suture patterns, cranial, experimental alterations of. By F. G. Girgis and J. J. Pritchard, 573
- Symons, N. B. B. Fixation of developing teeth, 583
- Szabo, G. Tyrosinase in melanocytes of normal, caucasian epidermis and numerical relationship of tyrosine-positive and dopa-positive cells, 593
- Tectal projection to reticular formation in cat. By G. W. Pearce and P. Glees, 599
- Tecto-spinal tract, crossed, in cat, termination of. By G. W. Pearce, 565
- Teeth, developing, fixation of. By N. B. B. Symons, 583
- Testis, rat, intravital staining of. By J. S. Baxter, 596
- Tobias, P. V. Premeiotic mitosis: a new type of cell-division in mammals, 570
- Tomlinson, J. W. D. Extra-dural vertebral venous systems in mammals, 575
- Tonge, C. H. Remnants of human odontogenic and adjacent oral epithelium, 584
- Torr, J. B. D. and Dixon, A. D. Determination of chromosomal sex and clinical application, 602
- Oral smear determination of chromosomal sex, 581
- Torr, J. B. D. *See* Dixon, A. D. joint authors, 582, 601
- Touch corpuscles, digital. By N. Cauna, 567
- Turner, C. J. *See* Asscher, A. W., joint authors, 577
- *See* Harrison, R. J., joint authors, 574
- Turner, P. *See* Bunn, D. I. G., joint authors, 562
- Tympanic cavity, mammalian, evolution of. By C. C. D. Shute, 572
- Tyrosinase, in melanocytes. By G. Szabo, 593
- Uterus, pregnant, cells in spiral arteries of. By J. D. Boyd and W. J. Hamilton, 595
- Uterus, rat, post-partum, vascular pattern of. By A. Young, 596
- Vaginal development. By D. Bulmer, 570
- Vaginal epithelium, human, sulphhydryl and disulphide groups in. By A. W. Asscher, C. J. Turner and C. H. de Boer, 577
- Vascular pattern of post-partum uterus of rat. By A. Young, 596
- Vein, oblique of left atrium and left precaval, in mammals. By T. S. King and J. B. Coakley, 601
- Venography, splenic, in rat. By J. L. Braithwaite and D. J. Adams, 596
- Vertebral venous systems, extra-dural, in mammals. By J. W. D. Tomlinson, 575
- Walls, E. W. and Gould, R. P. Epithelium of adult anal canal, 561
- Weddell, A. G. M. *See* Oppenheimer, D. R., joint authors, 579
- Weddell, G., Palmer, Elizabeth and Schofield, G. C. Leprosy as aid in investigation of neurohistology of skin, 599
- Wells, L. H. Neolithic remains from Long Barrow, 580
- Wilson, C. P. Bilateral fistulae of nasopharynx in living adult, 579
- Woollam, D. H. M. *See* Millen, J. W., joint authors, 567
- Wyburn, G. M. and Bacsich, P. Uptake of ³⁵S in cartilage homografts, 563
- Wyburn, G. M. *See* Bacsich, P., joint authors, 583
- Xenopus*, development of spinal cord in. By A. Hughes, 590
- Yoffey, J. M. *See* Pathak, V. B., joint authors, 568
- Young, A. Vascular pattern of post-partum rat uterus, 596

